Variability in conversion of β-carotene to vitamin A in men as measured by using a double-tracer study design1–3

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 (D₆) retinyl acetate (all-trans-19,19,19,19',19'-[²H₆]retinyl acetate) and then with 37 μmol D₆ β-carotene (19,19,19,19',19'-[²H₆]β-carotene) 1 wk later. Doses were taken with breakfasts containing 16 g fat. We measured D₆ retinol, D₆ β-carotene, and trideuterated (D₃) retinol (derived from D₆ β-carotene) concentrations in plasma. Areas under the plasma concentration × time since dosing curves (AUCs) were determined for D₆ retinol, D₆ β-carotene, and D₃ retinol.

Results: All men had detectable D₆ retinol concentrations in plasma. The mean (±SE) absorption of D₆ 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 D₆ β-carotene and D₃ retinol that we could measure. The mean absorption of D₆ 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 retinol, 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 (D₄) 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 D₃ 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 (D₆) β-carotene and a breakfast of 11 g fat, the mean activity was 0.8 mol trideuterated (D₃) retinol (derived from D₆ β-carotene) to 1 mol D₆ β-carotene (7). With breakfasts that provided 30 g fat, Tang et al (15) gave a dose of 30.6 μmol octadeuterated (D₈) retinyl acetate (in oil) in between a large and a small dose of D₆ β-carotene.

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\(\beta\)-carotene (235 compared with 11.2 \(\mu\)mol, each in oil) to a woman. They found the vitamin A activity of \(\beta\)-carotene to be 0.5 mol retinol to 1 mol \(\beta\)-carotene with the small dose of \(D_6\) \(\beta\)-carotene and only 0.03:1 with the large dose. Therefore, it appears that the vitamin A activity of \(\beta\)-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 \(\beta\)-carotene was recently reset to 0.94 mol retinol to 1 mol \(\beta\)-carotene in oil, 0.156 mol retinol to 1 mol \(\beta\)-carotene in food, 0.078 mol retinol to 1 mol \(\alpha\)-carotene in food, and 0.081 mol retinol to 1 mol \(\beta\)-cryptoxanthin in food (16). Men show a smaller rise in plasma \(\beta\)-carotene concentrations than do women when given similar oral doses of \(\beta\)-carotene (12). Male rats require \(\approx\)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 \(\beta\)-carotene (18), and the magnitude of bioconversion of \(\beta\)-carotene to retinol in men has not been studied with a double-label approach. Therefore, we determined the fates of approximately 0.081 mol retinol to 1 mol \(\beta\)-carotene (the provitamin A that is converted to \(D_7\) 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 SCAontrn version of the Block 92 Food Frequency Questionnaire (Block Dietary Data Systems, Berkeley, CA). The men ranged in age from 25 to 40 y (\(\pm\)6 y), had body weights from 55 to 110 kg (73 \(\pm\)16 kg), and had body mass indexes (in kg/m\(^2\)) from 19 to 31 (23 \(\pm\)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 \(\geq\)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 NNESSy, 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 \(\mu\)mol \(\beta\)-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 \(\mu\)mol 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 \(\mu\)mol (171 RE) \(\beta\)-carotene every other day at breakfast. These supplements were given to stabilize the plasma concentrations of total retinol and \(\beta\)-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 \(\mu\)mol crystalline \(D_6\) retinyl acetate with 250 mL milk (2% fat). Thirty (\(\pm\)5) minutes later, a breakfast containing 11 g fat was served. Similarly, on day 23, each man swallowed an additional gelatin capsule containing 37 \(\mu\)mol crystalline \(D_6\) \(\beta\)-carotene with 250 mL milk (2% fat). Thirty (\(\pm\)5) minutes later, a breakfast containing 11 g fat was served. Therefore, the \(D_6\) retinyl acetate and the \(D_6\) \(\beta\)-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 \(D_7\) retinol from provitamin A (cleavage of the \(D_6\) \(\beta\)-carotene). The mass of a supplemental dose of \(\beta\)-carotene (in mg) necessary to meet the vitamin A requirement of men is approximately twice that of retinol (12). Therefore, 30 \(\mu\)mol \(D_6\) retinyl acetate and 37 \(\mu\)mol \(D_6\) \(\beta\)-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 \(D_6\) \(\beta\)-carotene dose. The blood sample drawn at 168 h after the \(D_6\) retinyl acetate dose served as the time 0 sample for the \(D_6\) \(\beta\)-carotene dose. The men were in the fasting state when blood was drawn \(\approx\)24 h since dosing with \(D_6\) retinyl acetate and \(D_6\) \(\beta\)-carotene. Plasma was isolated and aliquots were placed in amber vials that were sealed under argon and stored at \(-75^\circ\)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 \(\mu\)mol \(\beta\)-carotene, 2.6 \(\mu\)mol \(\alpha\)-carotene, 0.2 \(\mu\)mol \(\beta\)-cryptoxanthin, 2.6 \(\mu\)mol lutein and zeaxanthin, and 1.2 \(\mu\)mol lycopene (Carotenoid Complex; GNLD, San Jose, CA). Each subject was then released from the MRU with 12 additional capsuless of the carotenoid supplement and instructions to take 1/d for the next 12 d.
Isotopes and supplements

The D₆ retinyl acetate (all-trans-19,19,19,20,20-[²H₆]retinyl acetate) and D₆ β-carotene (19,19,19,19,20,20-[²H₆]β-carotene) were from Cambridge Isotope Laboratories (Andover, MA). The isotopic purity of the D₆ retinyl acetate was determined by gas chromatography–mass spectrometry to be 91% D₃ retinyl acetate, 6% pentadeuterated (D₂) retinyl acetate, 2% D₁ retinyl acetate, and 1% other forms. The isotopic purity of the D₆ β-carotene was determined by fast atom bombardment mass spectrometry to be 59% D₆ β-carotene, 34% D₃ β-carotene, 6% D₂ β-carotene, and 1% other forms (24).

The unlabeled vitamin A supplement (with 1250 IU, or 375 RE per capsule) was from Bronzo (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 D₆ retinol were obtained by multiplying the plasma retinol concentration by (plasma D₆ retinol/plasma D₆ retinol + 1); concentrations of D₃ β-carotene were calculated similarly. The D₀ 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 D₆ β-carotene to D₃ β-carotene that could be integrated was 0.05. The D₀ β-carotene refers to nondeuterated or unlabeled β-carotene.

Calculation, analysis, and presentation of data

Plasma concentrations of D₆ retinol, D₃ retinol, and D₆ β-carotene by time since dosing were calculated and plotted. The area under the plasma concentration × time since dosing curve (AUC) for D₆ retinol was integrated from 0 to 96 h after dosing with D₆ retinol. The plasma D₃ retinol AUC was also integrated from 0 to 96 h since dosing with D₆ β-carotene. The plasma D₆ β-carotene AUC was integrated from 0 to 504 h after dosing with D₆ β-carotene. The plasma D₆ retinol and D₆ β-carotene AUCs could be summed to reflect the total absorption of administered D₆ β-carotene. Plasma AUCs were calculated by using the trapezoidal approximation (26).

The final conversion ratio was adjusted for differences in doses (D₆ retinyl acetate compared with D₆ β-carotene) by multiplying the plasma ratio of the D₆ retinol AUC to the D₆ retinol AUC by 30/37 (the molar ratio of the doses of D₆ retinyl acetate to D₆ β-carotene). The ratio of the AUCs of D₆ retinol to D₆ β-carotene may also reflect conversion efficiency. A high ratio of the AUCs of D₆ retinol to D₆ β-carotene in the presence of a low D₆ β-carotene AUC might suggest efficient conversion, whereas a low ratio of the AUCs of D₆ retinol to D₆ β-carotene in the presence of a high D₆ β-carotene AUC might suggest efficient absorption. The fractional absorption of D₆ 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 D₆ β-carotene. They are listed in the tables as low responders. The AUCs of D₆ β-carotene and D₆ retinol in plasma from the low responders were so low that meaningful ratios of D₆ retinol to D₆ 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 68828 IU/d, with a mean ± (SE) of 19323 ± 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 35455 ± 12192 IU/d. This difference was due largely to subject 11, who reported taking a 16666-U 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
The correlation between 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

<table>
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<th>Group and subject</th>
<th>Vitamin A intake</th>
<th>β-Carotene intake</th>
<th>Plasma retinol</th>
<th>Plasma β-carotene</th>
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<td></td>
<td>IU/d</td>
<td>µmol/d</td>
<td>Day 15</td>
<td>Day 22</td>
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<td>µmol/L</td>
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<td>11.55 ± 4.65</td>
<td>2.09 ± 0.17</td>
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</table>

1 During the study, meals were of natural foods adequate in vitamin A but low in carotene (1.07 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).

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 D₆ retinol, D₆ β-carotene, and D₁ retinol in 2 subjects (4 and 8, note the difference in the scale of the y axis) is shown in Figure 1. D₆ retinol rose promptly and peaked between 6 and 15 h after the D₆ retinyl acetate was administered. Of all 11 men, subject 4 had the second highest D₆ retinol concentration, whereas subject 8 had the lowest. The pattern of the plasma D₆ retinol concentration × time since dosing plot was generally similar to what we expected from our previous study of women (7).

The D₁ retinol concentration also rose promptly and peaked between 6 and 15 h after dosing with D₆ β-carotene. In general, the pattern of the plasma D₁ retinol concentration × time since dosing plot was similar to that of D₆ retinol. However, because the concentrations of D₁ retinol were low, they could only be measured in blood drawn during the first 4 d after dosing with D₆ β-carotene. The shortness of time that preceded the rise in D₆ retinol and D₆ retinol suggests that both are contributed by retinyl esters made in enterocytes during absorption.

The plasma D₆ β-carotene concentration × time since dosing curve had 2 peaks, the first at ≈10 h and the second at ≈4 d after dosing with D₆ 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 D₆ β-carotene AUC, whereas subject 8 had the lowest. Subject 8 had higher plasma D₆ β-carotene than D₆ retinol, yet a D₆ β-carotene to D₆ retinol conversion similar to that of subject 4 on the basis of the ratio of the AUCs for D₆ retinol to D₆ retinol.

The AUCs for plasma D₆ retinol, D₁ retinol, and D₆ β-carotene are summarized in Table 2. All subjects showed a measurable AUC for plasma D₆ retinol after ingesting the oral dose of D₆ retinyl acetate. The overall mean AUC for plasma D₆ retinol was 9.62 ± 2.19 µmol·h/L (range: 0.38–23.11 µmol·h/L). The difference in the mean AUC for D₆ retinol between the responder and low-responder groups was not significant.

The mean plasma D₆ 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 D₆ β-carotene (>0.01 µmol·h/L). These 6 men had a mean AUC for plasma D₆ β-carotene of 15.48 ± 4.79 µmol·h/L. The remaining 5 men (subjects 2, 6, 9, 11, and 12) had plasma D₆ 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 D₆ retinol (>0.001 µmol·h/L). They were the same men with a measurable AUC for plasma.
D₆ β-carotene. These 6 men had a mean D₃ 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 D₃ 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 D₆ β-carotene response.

The mean plasma D₃ retinol AUC was smaller than the mean plasma D₆ retinol AUC (P < 0.0014). Finally, the correlation between the AUCs for plasma D₃ retinol and those for plasma D₆ retinol were not significant (both for all subjects and for responders only, data not shown).

The ratio of the plasma AUCs of D₃ retinol to D₆ retinol reflects the yield of D₃ retinol from the D₆ β-carotene dose. The yield of D₃ retinol from D₆ β-carotene is relative to the yield of D₆ retinol from D₆ retinyl acetate. The mean ratio of D₃ retinol to D₆ 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 D₆ 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 D₆ β-carotene was too low in the responder group to be measured.

The strong correlations between the AUCs for plasma D₃ retinol and for its parent compound D₆ 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 framework.

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**TABLE 2**

Area under the plasma concentration × time curve (AUC) for hexadeuterated (D₆) retinol (derived from administered D₆ retinyl acetate), D₆ β-carotene, and trideuterated (D₃) retinol (derived from administered D₆ β-carotene) and D₆ β-carotene absorption data, by response group

<table>
<thead>
<tr>
<th>Group and subject</th>
<th>D₆ retinol (0–96 h AUC)</th>
<th>D₆ retinol (0–96 h AUC)</th>
<th>D₃:D₆ retinol</th>
<th>D₆ β-carotene (0–504 h AUC)</th>
<th>D₆ β-carotene absorption</th>
<th>D₆ retinol:D₆ β-carotene</th>
<th>D₆ retinol:D₆ β-carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.82</td>
<td>0.200</td>
<td>0.0891</td>
<td>3.04</td>
<td>0.804</td>
<td>0.066</td>
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</tr>
<tr>
<td>4</td>
<td>16.14</td>
<td>1.020</td>
<td>0.0512</td>
<td>30.00</td>
<td>7.939</td>
<td>0.034</td>
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</tr>
<tr>
<td>5</td>
<td>23.11</td>
<td>0.750</td>
<td>0.0263</td>
<td>29.04</td>
<td>7.687</td>
<td>0.026</td>
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</tr>
<tr>
<td>7</td>
<td>5.03</td>
<td>0.570</td>
<td>0.0919</td>
<td>11.29</td>
<td>2.988</td>
<td>0.050</td>
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</tr>
<tr>
<td>8</td>
<td>0.38</td>
<td>0.023</td>
<td>0.0495</td>
<td>4.44</td>
<td>1.176</td>
<td>0.005</td>
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<tr>
<td>10</td>
<td>14.54</td>
<td>0.290</td>
<td>0.0162</td>
<td>15.08</td>
<td>3.990</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>x ± SE</td>
<td>10.17 ± 3.72</td>
<td>0.476 ± 0.152</td>
<td>0.0540 ± 0.0128</td>
<td>15.48 ± 4.79</td>
<td>4.097 ± 1.208</td>
<td>0.033 ± 0.009</td>
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</tr>
<tr>
<td>Low responders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>13.15</td>
<td>0.001</td>
<td>0.0001</td>
<td>0.01</td>
<td>0.001</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7.48</td>
<td>0.001</td>
<td>0.0001</td>
<td>0.01</td>
<td>0.001</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.79</td>
<td>0.001</td>
<td>0.0010</td>
<td>0.01</td>
<td>0.001</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>9.82</td>
<td>0.001</td>
<td>0.0001</td>
<td>0.01</td>
<td>0.001</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>13.61</td>
<td>0.001</td>
<td>0.0001</td>
<td>0.01</td>
<td>0.001</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>x ± SE</td>
<td>8.97 ± 2.33</td>
<td>0.001 ± 0.0003</td>
<td>0.0003 ± 0.0002</td>
<td>0.01 ± 0.001</td>
<td>0.001 ± 0.001</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>9.62 ± 2.19</td>
<td>0.260 ± 0.109</td>
<td>0.0296 ± 0.0108</td>
<td>8.44 ± 3.49</td>
<td>2.235 ± 0.925</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

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 D₃ retinol AUC (0–96 h)/D₆ 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).
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 D₆ retinyl acetate and D₆ β-carotene 1 wk apart so that we could determine the response to ingested D₆ retinol as a reference and to ensure that it did not interfere with absorption of the D₆ β-carotene. Individual isopomers of retinol were measured simultaneously by selected ion monitoring mass spectrometry. In future studies, both the D₆ retinyl acetate and the D₆ 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 D₆ β-carotene also had low plasma D₁ 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 D₁ retinol and for plasma D₆ 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 D₁ retinol and for its parent compound D₆ 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 D₆ retinol that enabled a D₆ retinol AUC to be calculated for each subject (Table 2 and Figure 1). The positive correlation between the plasma D₆ retinol AUC and the plasma D₆ β-carotene AUC, especially within the responder group, was expected because the absorption of D₆ retinyl acetate and D₆ β-carotene share common mechanisms.

The nonsignificant correlation between plasma D₆ 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 D₆ 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 D₆ 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 D₆ 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 D₆ β-carotene and the smallest biocconversion to D₆ 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 down-regulated 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 D₆ 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 Δ9 retinyl acetate and Δ9 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 Δ9 β-carotene AUC response (responders) had a plasma Δ9 retinol AUC response. This indicates that low responders might have cleaved the Δ9 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 [14C]β-carotene could be added to the dosing cocktail for a reliable value for β-carotene absorption by mass balance (38).

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REFERENCES


