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**A Simple Plasma Retinol Isotope Ratio Method for Estimating  $\beta$ -Carotene Relative Bioefficacy in Humans: Validation Using Model-Based Compartmental Analysis**<sup>1-3</sup>

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<sup>3</sup> Supplemental Table 1, Supplemental Methods 1 and 2, and Supplemental WinSAAM Deck will be available at the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at [jn.nutrition.org](http://jn.nutrition.org).

<sup>6</sup> Abbreviations used: AUC<sub>p</sub>, area under the plasma retinol isotope response curve; DT, delay time; FD<sub>p</sub>, fraction of dose in plasma; IRM, isotope reference method; L(I,J), fractional transfer coefficient; RIR, plasma retinol isotope ratio.

1 **Abstract**

2 **Background:** Provitamin A carotenoids are a significant source of dietary vitamin A for many  
3 populations. Thus, accurate and simple methods for estimating carotenoid bioefficacy are  
4 needed to evaluate the vitamin A value of test solutions and plant sources.  $\beta$ -Carotene  
5 bioefficacy is often estimated from the ratio of areas under plasma isotope response curves after  
6 subjects ingest labeled  $\beta$ -carotene and a labeled retinyl acetate reference dose (isotope  
7 reference method; IRM) but, to our knowledge, the method has not yet been evaluated for  
8 accuracy.

9 **Objective:** Objectives were to (1) develop and test a physiologically-based compartmental  
10 model that includes both absorptive and post-absorptive  $\beta$ -carotene bioconversion and (2) use  
11 the model to evaluate the accuracy of the IRM and a simple plasma retinol isotope ratio (RIR;  
12 labeled  $\beta$ -carotene-derived retinol / labeled reference dose-derived retinol in one plasma sample)  
13 for estimating relative bioefficacy.

14 **Methods:** We used model-based compartmental analysis (Simulation, Analysis and Modeling  
15 software) to develop and apply a model that provided known values for  $\beta$ -carotene bioefficacy.  
16 Theoretical data for ten subjects were generated by the model and used to determine bioefficacy  
17 by RIR and IRM; predictions were compared to known values. We also applied RIR and IRM to  
18 previously-published data.

19 **Results:** Plasma RIR accurately predicted  $\beta$ -carotene relative bioefficacy at 14 d or later. IRM  
20 also accurately predicted bioefficacy by 14 d, except that, when there was substantial post-  
21 absorptive bioconversion, IRM underestimated bioefficacy. Based on our model, 1 d predictions  
22 of relative bioefficacy include absorptive plus a portion of early post-absorptive conversion.

23 **Conclusion:** The plasma RIR is a simple tracer method that accurately predicts  $\beta$ -carotene  
24 relative bioefficacy based on analysis of one blood sample obtained at 14 d or longer after co-  
25 ingestion of labeled  $\beta$ -carotene and retinyl acetate. The method also provides information about

- 26 the contributions of absorptive and post-absorptive conversion to total bioefficacy if an additional  
27 sample is taken at 1 d.
- 28 Key words:  $\beta$ -carotene; bioconversion; bioefficacy; carotenoids; humans; isotope reference  
29 method; model-based compartmental analysis; retinoids; retinol isotope ratio; WinSAAM

## 30 **Introduction**

31 Provitamin A carotenoids, especially  $\beta$ -carotene, are an important dietary source of vitamin A,  
32 especially in low-income countries where vitamin A deficiency remains a public health problem  
33 (1). Available data, based mainly on results for  $\beta$ -carotene, indicate that the bioavailability and  
34 bioconversion of provitamin A carotenoids to vitamin A [i.e., bioefficacy (2)] are affected by many  
35 factors, including food matrix, preparation methods, genetics, age, etc. (3). Although several  
36 methods have been proposed and used to measure  $\beta$ -carotene bioefficacy (4, 5), none are ideal  
37 for use in the field; in addition, none have been verified by comparison to known reference  
38 values. Researchers need a simple, accurate, and feasible method to assess bioefficacy  
39 because of  $\beta$ -carotene's contribution to whole-body vitamin A stores and plasma retinol  
40 homeostasis.

41       Recently (6), we used model-based compartmental analysis to study plasma retinol  
42 kinetics and to estimate relative bioefficacy of  $\beta$ -carotene in healthy adults who had consumed  
43 stable isotope-labeled  $\beta$ -carotene and a reference dose of labeled retinyl acetate. As we  
44 analyzed the data, the disposition of the two plasma isotope response curves led us to  
45 hypothesize that  $\beta$ -carotene bioefficacy relative to the reference dose could be estimated from  
46 the ratio of the two isotopes in a single blood sample, analogous to the plasma dual isotope ratio  
47 method for determining cholesterol absorption that was developed and tested by Zilversmit (7)  
48 and adapted by Green and Green for vitamin A absorption (8). In fact, mean  $\beta$ -carotene relative  
49 bioefficacy calculated from the plasma retinol isotope ratio (RIR<sup>6</sup>) on d 2 was 12.5%, close to the  
50 values determined by modeling (13.5%) or by graphical methods based on areas under the  
51 plasma retinol tracer response curves ("area under the curve;" AUC<sub>p</sub>) (13.1%) (6). We found that  
52 similar plasma retinol ratio methods for estimating bioefficacy had been previously postulated by  
53 Hickenbottom et al. (9) and Van Loo-Bouwman et al. (10), but the approach has apparently not  
54 received additional attention.

55 Here, we further investigate the plasma retinol isotope ratio for measuring  $\beta$ -carotene  
56 relative bioefficacy. We used model-based compartmental analysis (11) and the Simulation,  
57 Analysis and Modeling software (12) to verify the accuracy of the technique by first developing  
58 and then applying a compartmental model that would predict assigned values for  $\beta$ -carotene  
59 bioefficacy over a physiologically-reasonable range (5). We confirmed that our physiologically-  
60 based model predicted these assigned bioefficacies and then used the model to generate  
61 corresponding plasma isotope response data. Finally, we used the simulated plasma data to  
62 calculate relative bioefficacy by the plasma RIR and the isotope reference method [IRM (13)] and  
63 compared predictions to the known values. The IRM, in which the ratio of areas under the  
64 plasma isotope response curves after subjects ingest doses of labeled  $\beta$ -carotene and retinyl  
65 acetate, has been previously used to estimate  $\beta$ -carotene relative bioefficacy by several  
66 investigators, as reviewed by Tang (13). In addition to providing a way to evaluate the accuracy  
67 of the two methods, our model development and interpretation also provided insights into the  
68 complex processes and time course of the bioconversion of  $\beta$ -carotene to retinol, including its  
69 absorptive (intestinal) and post-absorptive (extra-intestinal) components. We show that the  
70 plasma retinol isotope ratio provides a simple, accurate, and easily-used method for estimating  $\beta$ -  
71 carotene relative bioefficacy.

72

### 73 **Methods**

74 *Subjects.* We selected ten subjects (see next paragraph) from previously-published studies in  
75 which participants received oral doses of stable isotope-labeled  $\beta$ -carotene and a labeled retinyl  
76 acetate reference dose. In one case (14), the reference dose was ingested 3 d after the dose of  
77 labeled  $\beta$ -carotene; plasma retinol tracer responses were measured for 56 ( $\beta$ -carotene-derived  
78 retinol) and 53 d (for retinyl acetate-derived retinol);  $\beta$ -carotene relative bioefficacy was estimated  
79 using the IRM. Subsequently, data for labeled plasma retinol derived from the retinyl acetate  
80 dose were modeled by Cifelli et al. (15) using WinSAAM v. 3.3.0 (12) to determine kinetic

81 parameters for retinol metabolism. In the second case (16), subjects ingested simultaneous oral  
82 doses of labeled  $\beta$ -carotene and retinyl acetate; plasma tracer responses were measured for 14  
83 d; Green et al. (6) retrospectively modeled plasma retinol data for the two labels and used  
84 WinSAAM to estimate  $\beta$ -carotene relative bioefficacy for subjects whose results indicated little to  
85 no post-absorptive  $\beta$ -carotene bioconversion.

86 For the current work, our sample (n=10) includes individuals with a wide range of total  
87 body vitamin A stores (66 – 2500  $\mu$ mol; **Supplemental Table 1**) and known vitamin A kinetics.  
88 We selected six subjects from the study of Cifelli et al. (15) [including the ones from Tang et al.  
89 (14) reported to have the lowest, average, and highest conversion of  $\beta$ -carotene] plus four  
90 subjects from Green et al. (6). Simulated data based on both published (6, 15) and assigned  
91 kinetic parameters for these individuals were used to advance understanding of  $\beta$ -carotene  
92 bioconversion and to evaluate the accuracy of the IRM and the newly-described plasma RIR for  
93 estimating  $\beta$ -carotene relative bioefficacy. We deemed ten to be an adequate number of subjects  
94 because it allowed for inclusion of a wide range of bioefficacies with differing contributions from  
95 absorptive and early/late post-absorptive bioconversion. Since we needed to have known values  
96 for bioefficacy in order to test the accuracy of predictions by the two methods, we assigned  
97 known, physiologically-reasonable values for total bioefficacy, including the absorptive and post-  
98 absorptive components, to each subject (see later).

99

100 *Compartmental models.* We used the six-compartment model presented by Green et al. (6)  
101 (**Figure 1A**) to describe the metabolism of retinyl acetate-derived retinol. In Figure 1A,  
102 components 1 to 4 represent the processing of dietary preformed vitamin A until it reaches the  
103 liver and is secreted into plasma compartment 5 as retinol bound to retinol-binding protein; retinol  
104 in the plasma pool exchanges with vitamin A in the storage pool (compartment 6), which is also  
105 the site of irreversible loss from the system.



106           Next, we developed a partially-parallel model (Figure 1B) that describes trafficking of  
107 labeled retinol derived from the conversion of absorbed  $\beta$ -carotene during both absorptive  
108 (intestinal) and early plus late post-absorptive (extra-intestinal) phases. A “partially-parallel  
109 model” means that we used the same values for kinetic parameters in the model for  $\beta$ -carotene-  
110 derived retinol as we used in the model for retinyl acetate-derived retinol when we were  
111 describing biochemical and physiological processes that one logically assumes to be identical for  
112 the two labels. It should be emphasized that this work focuses on retinol derived from the labeled  
113  $\beta$ -carotene dose (i.e., on  $\beta$ -carotene that is converted to retinol and secreted into plasma after  
114 conversion). For the absorptive / intestinal pathway (Figure 1B, unshaded components), the  
115 model was parallel to the one in Figure 1A; that model had been developed (6) for subjects  
116 whose data indicated little to no post-absorptive conversion of absorbed  $\beta$ -carotene to retinol.  
117 For the post-absorptive / extra-intestinal pathway (Figure 1B, shaded components), we used  
118 aspects of the model presented by Novotny et al. (17) as well as other information in the literature  
119 (18-20). In Figure 1B, components 11 to 13 represent the processes of digestion and absorption  
120 of  $\beta$ -carotene; compartment 11 is the site of splitting of the  $\beta$ -carotene dose into the fraction  
121 eventually converted to retinol (that which is transferred to compartment 12) versus that which  
122 does not contribute to bioefficacy. Bioefficacy was partitioned into absorptive and post-absorptive  
123 components at delay component 13 as follows. For the intestinal bioconversion pathway,  
124 absorbed  $\beta$ -carotene is converted to retinol and packaged in chylomicrons, and the retinol  
125 (primarily retinyl esters) is transferred to compartment 14. For the post-absorptive pathway,  
126 chylomicron  $\beta$ -carotene is transferred from component 13 to compartment 21, which represents  
127 tissue uptake (both extrahepatic and hepatic) of  $\beta$ -carotene from chylomicrons or chylomicron  
128 remnants. This  $\beta$ -carotene can be converted to retinol in tissues (compartment 24), representing  
129 early post-absorptive conversion (solid-line arrow), or it may be delayed by passing from  
130 compartment 21 through components 22 and 23 (possibly plasma lipoproteins or adipocyte lipid  
131 droplets; see Discussion) before conversion to retinol and passage into compartment 24,

132 representing late post-absorptive conversion (dashed-line arrows). Similar to the models  
133 developed by Green et al. (21) and Novotny et al. (17), retinol in compartment 24 is then either  
134 secreted directly into plasma compartment 15 bound to retinol-binding protein or esterified for  
135 storage in compartment 16.

136

137 *Assignment of known values for  $\beta$ -carotene bioefficacy, including both absorptive and post-*  
138 *absorptive components.* In order to obtain known reference values for bioefficacy against which  
139 we could evaluate the RIR and IRM, we chose ten values for  $\beta$ -carotene relative bioefficacy  
140 ranging from 10 to 50%, reflecting the variability reported in the literature (5); then we assigned a  
141 value to each subject. Assignments were made based on individual total body vitamin A stores  
142 (Supplemental Table 1); the results of Green et al. (6), which indicated that the subjects in that  
143 study had little post-absorptive conversion; and the results of Tang et al. (14), from which we  
144 selected the three subjects that were reported to be the lowest, average, and highest converters  
145 of  $\beta$ -carotene. Each level of relative bioefficacy was then partitioned into hypothetical but  
146 physiologically-reasonable values for absorptive (7 – 40%) and post-absorptive (3 – 20%)  
147 components. For example, for subject 1, we assigned a total relative bioefficacy of 10% and  
148 attributed 7% bioefficacy to intestinal bioconversion and 3% to extra-intestinal processes.  
149 Additionally, each value for post-absorptive conversion was partitioned into early and late  
150 components (e.g., 2.4% early and 0.6% late for subject 1). As described in **Supplemental**  
151 **Methods 1**, we simulated the model for each individual to verify that it accurately predicted the  
152 assigned values for the various components of bioefficacy.

153

154 *Use of compartmental analysis to generate plasma isotope data for retinyl acetate- and  $\beta$ -*  
155 *carotene-derived retinol.* To generate plasma retinol tracer response data [fraction of dose in  
156 plasma ( $FD_p$ ) versus time] for the retinyl acetate reference dose, we used the kinetic parameters

157 reported for the ten subjects (Supplemental Table 1), including an absorption efficiency of 75%,  
 158 as inputs in the compartmental model presented by Green et al. (6) (Figure 1A). Time points  
 159 (n=21; 0, 3, 6, 9, and 11 h, then daily for 9 d, and weekly from 14 to 56 d) for the simulation were  
 160 based on the study of Tang et al. (14), in which extensive blood sampling had been done to  
 161 provide the detailed plasma tracer kinetic curves needed for model-based compartmental  
 162 analysis.

163 Next, to generate corresponding data for  $\beta$ -carotene-derived retinol, we fixed parameters  
 164 in the model shown in Figure 1B at the values presented in Supplemental Table 1. These  
 165 included model parameters described in **Supplemental Methods 2** that generated the assigned  
 166 values for total, absorptive, and post-absorptive (early and late) bioefficacy. Then, data for  $FD_p$   
 167 versus time (3 h to 56 d) were simulated to generate plasma tracer response profiles for retinol  
 168 derived from the  $\beta$ -carotene dose for each of the ten subjects at the various levels of bioefficacy.  
 169 For each subject, simulations resulted in plasma data for labeled retinol derived from three  
 170 sources: the retinyl acetate reference dose, absorptive (intestinal) conversion of  $\beta$ -carotene to  
 171 retinol, and post-absorptive (extra-intestinal, early and late) conversion of  $\beta$ -carotene to retinol.  
 172 Datasets for  $FD_p$  versus time were used to calculate  $\beta$ -carotene relative bioefficacies based on  
 173 plasma isotope ratios and areas under the curves as described in the next sections.

174

175 *Calculation of  $\beta$ -carotene relative bioefficacy from the plasma retinol isotope ratio.* Relative  
 176 bioefficacy was calculated for the datasets described above based on retinol isotope ratios in  
 177 plasma at specific times using Equation 1:

178

$$179 \quad \text{RIR relative bioefficacy (\%)} = \left( \frac{FD_p \text{ for labeled } \beta\text{-carotene-derived retinol}}{FD_p \text{ for labeled retinyl acetate-derived retinol}} \right) \times 100 \quad (1)$$

181

182 The plasma RIR was calculated using  $FD_p$  data in molar retinol activity equivalents (1  $\mu\text{mol}$   $\beta$ -  
183 carotene = 2  $\mu\text{mol}$  retinol) at times from 1 to 56 d after dose administration.

184

185 *Calculation of  $\beta$ -carotene relative bioefficacy using the isotope reference method.* Relative  
186 bioefficacy was calculated for the same data using the IRM as applied by Tang et al. (14) and  
187 Green et al. (6) as shown in Equation 2:

188

$$\begin{aligned} \text{IRM relative bioefficacy (\%)} = & (\text{AUC}_p \text{ for labeled } \beta\text{-carotene-derived retinol} / \\ & \text{AUC}_p \text{ for labeled retinyl acetate-derived retinol}) \times 100 \end{aligned} \quad (2)$$

191

192 The trapezoid rule was applied to  $FD_p$  data using GraphPad Prism v. 7.0 for Windows (GraphPad  
193 Software Inc., La Jolla, CA) to determine  $AUC_p$  from time zero to the end of the experiment (56 d)  
194 or to earlier times.

195

196 *Comparison of calculated values for relative bioefficacy to known values.*  $\beta$ -Carotene  
197 bioefficacies calculated for each individual using the plasma RIR (Equation 1) and the IRM  
198 (Equation 2) were compared to the known (assigned) values for total, absorptive, and post-  
199 absorptive (both early and late) bioefficacy in order to evaluate the accuracy of the two  
200 techniques.

201

202 *Application of the plasma retinol isotope ratio and the isotope reference method to previously-*  
203 *published kinetic data.* We expanded the application of the plasma RIR (Equation 1) and IRM  
204 (Equation 2) to estimate  $\beta$ -carotene relative bioefficacy for the group of healthy young adults  
205 ( $n=30$ ) that had been previously studied by Green et al. (6). In that paper, relative bioefficacies  
206 were calculated using the model-based  $AUC_p$  method in WinSAAM [i.e., (plasma residence time  
207 for  $\beta$ -carotene-derived retinol divided by plasma residence time for retinyl acetate-derived retinol)]

208 x 100]. These values were compared in (6) to estimates calculated using the IRM at 14 d; results  
209 for the plasma RIR at d 2 were also mentioned in (6). For the current analysis, relative  
210 bioefficacy was calculated using Equations 1 and 2 at 1, 2, 7, and 14 d.

211

212 *Statistical analysis.* Figures were prepared and linear regression analysis was done using  
213 GraphPad Prism v. 7.0 for Windows. To identify time-dependent changes in bioefficacy  
214 calculated using the RIR and IRM for subjects from (6), we used repeated measures ANOVA  
215 (JMP® Pro v. 12.1.0; SAS Institute Inc., Cary, NC) and to assess within-subject differences in  
216 predictions, we used matched pairs analysis (JMP® Pro v. 12.1.0).  $P < 0.05$  was considered  
217 significant.

218

## 219 **Results**

220 *Confirmation of assigned (known) values for  $\beta$ -carotene bioefficacy.* We used model simulations  
221 as described in Supplemental Methods 1 to confirm that our physiologically-based compartmental  
222 model (Figure 1B) accurately predicted the assigned values for the various components of  
223 bioefficacy (total, absorptive, and early and late post-absorptive) for each individual. As shown in  
224 **Figure 2** for subject 5, the simulation correctly predicted the assigned values for total (actual, not  
225 relative) bioefficacy (18.75%), absorptive bioefficacy (15%), and post-absorptive bioefficacy  
226 (3.75%), as well as the components of post-absorptive (early at 1.69% and late at 2.06%). For  
227 this subject, values for all components plateaued by 5 d and were time invariant after that. Model  
228 simulations also precisely predicted assigned (known) values for bioefficacy, including all forms of  
229 bioconversion, for the other nine subjects (data not shown).

230

231 *Plasma isotope response data/curves for the retinol reference dose and for  $\beta$ -carotene-derived*  
232 *retinol.* Using the model shown in Figure 1A and the kinetic parameters from Cifelli et al. (15) and  
233 Green et al. (6) that are presented in Supplemental Table 1, we simulated tracer data for plasma

234 retinol derived from the retinyl acetate reference dose versus time. As shown in **Figures 3A** and  
235 **B** (solid lines) for subjects 2 and 8, respectively, plasma retinol tracer peaked ~13 h after dosing  
236 and then gradually fell as retinol was distributed to tissues. The bend in the curves at ~7 d  
237 indicates recycling of tracer back to plasma. By 10 d post-dosing, the curves entered a terminal  
238 slope, equivalent to the apparent system fractional catabolic rate (~3%/d for these two subjects).  
239 Similar patterns were observed for the retinyl acetate-derived retinol curves for the other subjects  
240 (data not shown).

241 Also presented in Figures 3A and B are the simulated plasma tracer response data for  $\beta$ -  
242 carotene-derived retinol (dashed lines) when relative bioefficacy was fixed at 14% (10%  
243 absorptive and 4% post-absorptive; subject 2) or at 40% (30% absorptive and 10% post-  
244 absorptive; subject 8). Note that, at the higher (versus lower) level of total  $\beta$ -carotene bioefficacy,  
245 the curve for  $\beta$ -carotene-derived retinol is closer to the one for retinol from the reference dose.  
246 For all subjects (data not shown), before any late post-absorptive conversion occurred, curves for  
247  $\beta$ -carotene-derived retinol were similar to those for retinyl acetate-derived retinol (i.e., the two  
248 curves appeared to be parallel, as illustrated in Figures 3A and B). At the time set for late post-  
249 absorptive conversion (delay component 22; Figure 1B), which was varied from 2 to 5 d across  
250 subjects (Supplemental Table 1), there was an increased influx of tracer into plasma as newly-  
251 formed labeled retinol was secreted into compartment 15. At the higher levels of post-absorptive  
252 conversion (7 to 20%), the increased input was evident as a discernible hump in the curves for  $\beta$ -  
253 carotene-derived retinol (as illustrated by the data in Figure 3B), whereas at the lower levels of  
254 post-absorptive conversion (3 to 5%), this influx caused only subtle changes in the curves (as in  
255 Figure 3A). Plasma retinol tracer stabilized after late post-absorptive conversion and the curves  
256 entered a terminal slope by ~10 d, becoming parallel to the curve for retinyl acetate-derived  
257 retinol.

258 Next, to examine the two components of bioefficacy separately, we used model-based  
259 compartmental analysis to simulate the expected plasma isotope response curves for  $\beta$ -carotene-

260 derived retinol formed from absorptive versus post-absorptive conversion. **Figure 4** shows these  
261 simulations, along with the curve for retinol derived from the reference dose, for subject 5; see  
262 also **Supplemental WinSAAM Deck**. As was the case for all subjects (data not shown), the  
263 curve for absorptive bioconversion (dash-dot line in Figure 4) peaked at ~13 h, similar to that for  
264 the retinyl acetate-derived retinol (solid line); curves were parallel from this point on. The curve  
265 for post-absorptive conversion (dotted line) showed two distinct peaks. The first peak occurred  
266 ~13 h after dosing, similar to that for the absorptive phase, representing early post-absorptive  
267 conversion, whereas the second peak occurred after late post-absorptive conversion (2.5 d). Our  
268 model simulation predicts that a portion of the retinol formed during early post-absorptive  
269 conversion enters plasma with the same time course as intestinally-produced retinol; see  
270 Discussion. When there is extensive late post-absorptive conversion, there is a hump in the  
271 composite curve for  $\beta$ -carotene-derived retinol (absorptive plus post-absorptive; dashed line).  
272 After ~10 d, the curves for  $\beta$ -carotene-derived retinol parallel that for the retinyl acetate-derived  
273 retinol for the duration of the experiment (56 d).

274  
275  *$\beta$ -Carotene relative bioefficacy calculated by the RIR and IRM.* Relative bioefficacies predicted  
276 by the plasma RIR (Equation 1) and the IRM (Equation 2) were compared to known (assigned)  
277 values for total, absorptive, and post-absorptive relative bioefficacy for the ten subjects (**Table 1**).  
278 For all subjects, the plasma RIR accurately predicted known values for total relative bioefficacy  
279 from 14 d to the end of the experiment (56 d); specifically, the RIR at either 14 or 56 d predicted a  
280 mean of 101% of the known values (range, 100 to 103%). Bioefficacy estimates calculated by  
281 the IRM predicted a mean of 92% (range, 74 to 99%) and 95% (range, 84 to 100%) of total  
282 bioefficacy at 14 and 56 d, respectively. For 8 of the 10 subjects, the IRM predictions were very  
283 close to the known values (96 and 98% of the total at 14 and 56 d, respectively)

284 We then compared the equations' predictions on d 1 to known values for absorptive  
285 bioefficacy, as has been done in earlier applications of the IRM when the prediction on 1 d has

286 been said to indicate intestinal bioconversion (14, 22). The d 1 plasma RIR overestimated the  
287 assigned values for absorptive bioconversion by a mean of 17% and the IRM overestimated them  
288 by 26% (Table 1), suggesting that the d 1 predictions included more than absorptive conversion.  
289 As shown in Figure 4 inset, at 1 d, the composite curve for  $\beta$ -carotene-derived retinol (dashed  
290 line) is higher than the curve for retinol derived from intestinal conversion (dash-dot line). This  
291 difference is attributable to a contribution from early post-absorptive conversion (see Discussion).  
292 Note that, when the plasma RIR and IRM were calculated using the appropriate simulated data  
293 for retinol derived from intestinal  $\beta$ -carotene conversion and the retinyl acetate reference dose,  
294 both methods predicted known values for absorptive bioefficacy at all times from 1 d on (data not  
295 shown). When known values for absorptive and early post-absorptive bioefficacy were added  
296 together using data in Table 1, the RIR at 1 d predicted a mean of 96% of the sum (range, 85 to  
297 103%); the linear regression equation was  $y = 0.95x + 1.8$  ( $R^2 = 0.99$ ), where  $y$  is the known  
298 value for absorptive plus early post-absorptive bioefficacy and  $x$  is the RIR on d 1. This indicates  
299 that, on average, ~4% of the retinol produced by early post-absorptive conversion appeared in  
300 plasma after d 1 and is included in the prediction of bioefficacy at 14 d. For the IRM, calculations  
301 on d 1 predicted 100% (range, 79 to 120%) of the sum of the absorptive plus early post-  
302 absorptive bioefficacy. Thus, in contrast to previous assumptions that d 1 predictions by the IRM  
303 represent absorptive bioconversion (14), our model predicts that d 1 data include not only the  
304 absorptive but also some of the early post-absorptive conversion of  $\beta$ -carotene to retinol.

305 To illustrate the changes in relative bioefficacy calculated by the RIR and IRM over time,  
306 we present in **Figure 5** results for subject 7 who was assigned 35% total relative bioefficacy,  
307 partitioned into 28% absorptive and 7% post-absorptive (2.1% early and 4.9% late). For this  
308 subject, the plasma RIR at 1 d predicted a relative bioefficacy of 30%, close to the known value  
309 for absorptive plus early post-absorptive bioefficacy (30.1%). After late post-absorptive  
310 conversion occurred (4.5 d for subject 7) and newly converted retinol derived from  $\beta$ -carotene  
311 entered plasma, the RIR was higher (i.e., the numerator in Equation 1 increased), peaking at 6.5



312 d at 48% (138% of the total). The value then fell and stabilized by 14 d at 35.5%, near the known  
313 value for total relative bioefficacy (35%). For all subjects, the peak in the RIR occurred between 4  
314 and 7 d post-dosing; by 14 d, the RIR stabilized near the known value for total relative bioefficacy.  
315 Similar trends versus time were observed for the other subjects (data not shown). These results  
316 show that the RIR is highly responsive to late post-absorptive conversion and needs time to  
317 stabilize; for these subjects, when late conversion occurred between 2 and 5 d, stabilization in the  
318 ratio was evident by 14 d.

319 In the case of the IRM, predictions for subject 7 (Figure 5) gradually increased following  
320 late post-absorptive conversion, averaging 92 and 95% of the total bioefficacy at 14 and 56 d,  
321 respectively. Similar trends were observed for the other subjects (data not shown). Overall, the  
322 IRM predictions at 14 or 56 d were close to the assigned values for total relative bioefficacy,  
323 except when there was substantial (late) post-absorptive conversion. Specifically, at the two  
324 highest levels of late post-absorptive bioconversion (subjects 6 and 10; Table 1), the IRM  
325 underestimated total relative bioefficacy by ~25% at 14 d and ~15% at 56 d.

326  
327 *Comparison of predictions of the plasma RIR and the IRM when applied to previously-published*  
328 *retinol kinetic and bioefficacy data.* We applied Equations 1 (RIR) and 2 (IRM) to previously-  
329 published data (6) for 30 adults for whom bioefficacy had been estimated using WinSAAM. Mean  
330 values for relative bioefficacy predicted by the RIR and IRM are plotted over time in **Figure 6**.  
331 Predictions of the two equations were similar at 1 and 2 d (~12%). For the plasma RIR,  
332 predictions were significantly higher on d 7 and d 14 (~15%) compared to d 1 and 2, indicating  
333 that some post-absorptive conversion of  $\beta$ -carotene to retinol (~3%) occurred between 2 and 7 d  
334 in some of these subjects, even though plasma isotope response curves for subjects in (6)  
335 suggested little to no post-absorptive conversion. Based on our theoretical work, we hypothesize  
336 that predictions of relative bioefficacy by the RIR at 1 and 2 d include all of the absorptive and  
337 most of the early post-absorptive bioconversion, and estimates at 7 and 14 d include the late

338 post-absorptive conversion as well. For the IRM, predictions were higher on 7 d versus d 1 and  
339 2, and higher still on 14 d (~13%), also suggesting some post-absorptive bioconversion (~1%).  
340 For individual comparisons, the RIR on d 14 predicted significantly higher values for relative  
341 bioefficacy compared to the IRM, by a mean difference of 2.3% bioefficacy ( $P < 0.0001$ ). Relative  
342 bioefficacy predicted by the RIR on d 14 ranged from 4.6 to 37.1% over all subjects, whereas for  
343 the IRM, 14 d predictions ranged from 4.5 to 30.7%. The maximum difference in relative  
344 bioefficacy values at 14 d versus 1 d was 7.7% for the RIR and 5.1% for the IRM. These results  
345 suggest that the RIR is more responsive than the IRM to (late) post-absorptive bioconversion,  
346 even when little occurs. Based on our theoretical analysis, we suggest that the RIR likely  
347 provides a more accurate estimate of  $\beta$ -carotene relative bioefficacy using data from a single  
348 blood sample obtained at 14 d or later.

349

## 350 **Discussion**

351 In this paper, we show (Table 1) that  $\beta$ -carotene relative bioefficacy is accurately estimated  
352 based on the ratio of two retinol isotopes in a single plasma sample (Equation 1) obtained at 14  
353 or more days after co-ingestion of labeled  $\beta$ -carotene and a labeled retinyl acetate reference  
354 dose. Such an approach had been previously suggested by Hickenbottom et al. (9) and Green et  
355 al. (6) but had not been evaluated or used. The single sample approach will make it more  
356 feasible to measure  $\beta$ -carotene relative bioefficacy in field settings, where it can be difficult to  
357 obtain multiple samples over time, as is done when using the isotope reference method (Equation  
358 2) to estimate relative bioefficacy (13). In addition, the single sample method will facilitate  
359 measuring bioefficacy in children, in whom repeated sampling is difficult. It is worth noting that  
360 investigators could also assess vitamin A status by collecting an additional blood sample at 4 or 5  
361 d and applying an appropriate retinol isotope dilution equation to the retinyl acetate-derived retinol  
362 data to predict individual vitamin A total body stores (23).

363 To our knowledge, this study is the first to evaluate the accuracy of methods for estimating  
364  $\beta$ -carotene relative bioefficacy. We used model-based compartmental analysis (11) and model  
365 simulations to verify predictions of known (assigned) values for bioefficacy (Figure 2) and then  
366 applied Equations 1 and 2 to calculate relative bioefficacy using simulated plasma retinol tracer  
367 response data. This approach is similar to the one we recently used to test the accuracy of an  
368 equation for estimating vitamin A total body stores at isotopic equilibrium (24). Here, in addition  
369 to demonstrating that the plasma RIR accurately predicts  $\beta$ -carotene relative bioefficacy at 14 d  
370 or later after dosing, we also showed that the IRM accurately predicts relative bioefficacy for  
371 some subjects (specifically, those with low levels of late post-absorptive bioconversion). It would  
372 be interesting for other investigators to now apply the RIR retrospectively to their previously-  
373 collected data from IRM studies to compare results for the two methods.

374 It should be emphasized that both the plasma RIR and IRM estimate bioefficacy *relative* to  
375 the absorption efficiency of the co-administered retinyl acetate reference dose. As methods are  
376 developed for measuring vitamin A absorption efficiency, it will be possible to correct estimates of  
377  $\beta$ -carotene relative bioefficacy by including the actual value for absorption efficiency of the  
378 reference dose (bioefficacy = relative bioefficacy  $\times$  reference dose absorption efficiency).

379 In addition to providing a way to evaluate the accuracy of methods for estimating  $\beta$ -  
380 carotene relative bioefficacy, compartmental modeling also led to insights into the time course  
381 and likely pathways for conversion of absorbed  $\beta$ -carotene to retinol. For provitamin A, previous  
382 work (14, 17, 22, 25) has suggested that some of the absorbed carotenoid is converted to retinol  
383 in the intestine and another portion is converted later (post-absorptive bioconversion) in tissues  
384 such as liver and adipose tissue. Starting with these assumptions, the model (Figure 1B)  
385 developed to generate plasma isotope response data for retinol derived from  $\beta$ -carotene included  
386 a site (delay component 13) for partitioning absorbed  $\beta$ -carotene destined for intestinal  
387 conversion (compartment 14) or for post-absorptive conversion (compartment 21). We  
388 hypothesize that  $\beta$ -carotene in chylomicrons taken up by the liver or extrahepatic tissues could be

389 converted to retinol (compartment 24) soon after uptake, representing the early post-absorptive  
390 phase. Alternatively,  $\beta$ -carotene could be transferred from compartment 21 to delay component  
391 22 and compartment 23, representing the late post-absorptive conversion pathway. The late  
392 post-absorptive route might involve  $\beta$ -carotene incorporation into very low density lipoproteins in  
393 the liver, with release of those lipoproteins into the circulation and subsequent metabolism to low-  
394 and high density lipoproteins (delay component 22), followed by uptake of plasma lipoprotein  $\beta$ -  
395 carotene by tissues in which post-absorptive bioconversion can occur. Alternatively, the late  
396 post-absorptive route might involve uptake of chylomicron  $\beta$ -carotene into (for example) adipose  
397 tissue and then partitioning into lipid droplets with subsequent conversion to retinol (compartment  
398 24). As shown in Figure 1B, this retinol can either be secreted into plasma compartment 15  
399 bound to retinol-binding protein or it can be esterified for storage in compartment 16.

400 Plasma isotope response curves like the one for  $\beta$ -carotene-derived retinol in Figure 3  
401 reflect the composite impact on retinol kinetics of all  $\beta$ -carotene bioconversion processes that  
402 result in the appearance of labeled retinol in plasma; methods like the RIR and IRM estimate  
403 relative bioefficacy using plasma retinol tracer data. Thus, if some absorbed  $\beta$ -carotene is  
404 converted locally in tissues to biologically active forms of vitamin A (26) or to apocarotenoids (27),  
405 those compounds never reach plasma as retinol and so these quantitatively minor pathways do  
406 not contribute to bioefficacy as currently defined (2).

407 As illustrated in Figure 4, one can use model simulations to look at the likely time course  
408 of the individual components of bioefficacy. This analysis revealed two distinct peaks in the curve  
409 for retinol derived from post-absorptive conversion: the first, corresponding to early post-  
410 absorptive conversion, occurs at the same time (~13 h after dose ingestion) as the peak in the  
411 curve for retinol generated by absorptive bioconversion; the second peak (at 2.5 d) corresponds  
412 to the late post-absorptive process. Depending on the extent of late post-absorptive conversion,  
413 there may be a discernible “hump” in the composite curve for  $\beta$ -carotene-derived retinol

414 (absorptive plus post-absorptive). This is evident in Figure 3B and can be seen in the results of  
415 other investigators (14, 17, 28).

416 Our current results suggest that retinol formed from early post-absorptive conversion  
417 enters plasma with the same time course as intestinally-produced retinol. Thus, based on our  
418 model, predictions of the plasma RIR at 1 d include retinol derived from absorptive conversion  
419 and some (probably most) of the early post-absorptive conversion; the early post-absorptive  
420 component might include some conversion of the labeled  $\beta$ -carotene dose that occurs in the  
421 intestine following subsequent meals. In earlier applications of the isotope reference method (14,  
422 22), d 1 results have been interpreted to represent intestinal bioconversion. Similar to results by  
423 Tang et al. (14) which indicated that 81% of the total  $\beta$ -carotene conversion to vitamin A had  
424 occurred by 1 d, our results indicate that for the RIR, 81% of the total relative bioefficacy was  
425 predicted at 1 d (86% for the IRM). By collecting a blood sample at 1 d as well as 14 d,  
426 researchers could use the RIR to estimate the contribution of absorptive- and early post-  
427 absorptive conversion to total relative bioefficacy.

428 Our data indicate that, after late post-absorptive conversion, the RIR transiently  
429 overestimates relative bioefficacy before stabilizing at the correct value for "total" bioefficacy by  
430 14 d. Time for ratio stabilization is also required for the plasma dual isotope ratio method used to  
431 determine cholesterol absorption (7) and vitamin A absorption (8). It is interesting to note that, if  
432 RIR predictions on d 14 for a given individual are substantially higher than those on d 1, this  
433 indicates that late post-absorptive bioconversion is an important contributor to total bioefficacy in  
434 that subject.

435 In conclusion, compared to graphical methods (e.g., the isotope reference method), the  
436 plasma retinol isotope ratio provides a simpler and more accurate method for estimating  
437 carotenoid relative bioefficacy following co-administration of isotope-labeled doses of  $\beta$ -carotene  
438 and retinyl acetate (reference dose). The plasma retinol isotope ratio method should be valid for  
439 use in both children and adults, and it can be used for test solutions (e.g., isotopes administered

440 in oil) or for intrinsically-labeled carotenoid in foods. The plasma RIR predicts  $\beta$ -carotene relative  
441 bioefficacy based on analysis of one blood sample collected 14 d or more after dosing.

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## References

1. Joint FAO/WHO Expert Consultation. Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation. 2<sup>nd</sup> ed. Geneva (Switzerland): World Health Organization; 2004.
2. West CE, Eilander A, van Lieshout M. Consequences of revised estimates of carotenoid bioefficacy for dietary control of vitamin A deficiency in developing countries. *J Nutr* 2002;132:2920S-6S.
3. Castenmiller JJM, West CE. Bioavailability and bioconversion of carotenoids. *Annu Rev Nutr* 1998;18:19-38.
4. van Lieshout M, West CE, van Breemen RB. Isotopic tracer techniques for studying the bioavailability and bioefficacy of dietary carotenoids, particularly  $\beta$ -carotene, in humans: a review. *Am J Clin Nutr* 2003;77:12-28.
5. Van Loo-Bouwman CA, Naber THJ, Schaafsma G. A review of vitamin A equivalency of  $\beta$ -carotene in various food matrices for human consumption. *Br J Nutr* 2014;111:2153-66.
6. Green MH, Ford JL, Oxley A, Green JB, Park H, Berry P, Boddy AV, Lietz G. Plasma retinol kinetics and  $\beta$ -carotene bioefficacy are quantified by model-based compartmental analysis in healthy young adults with low vitamin A stores. *J Nutr* 2016;146:2129-36.
7. Zilversmit DB. A single blood sample dual isotope method for the measurement of cholesterol absorption in rats. *Proc Soc Exp Biol Med* 1972;140:862-5.
8. Green MH, Green JB. Use of a plasma dual isotope ratio method to measure vitamin A absorption. *FASEB J*. 1997;11:A142 (abstract 825).
9. Hickenbottom SJ, Lemke SL, Dueker SR, Lin Y, Follett JR, Carkeet C, Buchholz BA, Vogel JS, Clifford AJ. Dual isotope test for assessing  $\beta$ -carotene cleavage to vitamin A in humans. *Eur J Nutr* 2002;41:141-7.
10. Van Loo-Bouwman CA, West CE, van Breemen RB, Zhu D, Siebelink E, Versloot P, Hulshof PJM, van Lieshout M, Russel FGM, Schaafsma G, Naber THJ. Vitamin A equivalency of  $\beta$ -



carotene in healthy adults: limitation of the extrinsic dual-isotope dilution technique to measure matrix effect. *Br J Nutr* 2009;101:1837-45.

11. Cifelli CJ, Green JB, Green MH. Use of model-based compartmental analysis to study vitamin A kinetics and metabolism. *Vitam Horm* 2007;75:161-95.

12. Wastney ME, Patterson BH, Linares OA, Greif PC, Boston RC. Chapter 5 (WinSAAM) in: *Investigating biological systems using modeling: strategies and software*. San Diego (CA): Academic Press; 1999.

13. Tang G. Techniques for measuring vitamin A activity from  $\beta$ -carotene. *Am J Clin Nutr* 2012;96:1185S-8S.

14. Tang G, Qin J, Dolnikowski GG, Russell RM. Short-term (intestinal) and long-term (postintestinal) conversion of  $\beta$ -carotene to retinol in adults as assessed by a stable-isotope reference method. *Am J Clin Nutr* 2003;78:259-66.

15. Cifelli CJ, Green JB, Wang Z, Yin S, Russell RM, Tang G, Green MH. Kinetic analysis shows that vitamin A disposal rate in humans is positively correlated with vitamin A stores. *J Nutr* 2008;138:971-7.

16. Oxley A, Berry P, Taylor GA, Cowell J, Hall MJ, Hesketh J, Lietz G, Boddy AV. An LC/MS/MS method for stable isotope dilution studies of  $\beta$ -carotene bioavailability, bioconversion, and vitamin A status in humans. *J Lipid Res* 2014;55:319-28.

17. Novotny JA, Dueker SR, Zech LA, Clifford AJ. Compartmental analysis of the dynamics of beta-carotene metabolism in an adult volunteer. *J Lipid Res* 1995;36:1825-38.

18. Berg T, Blomhoff R, Norum KR. Transfer of retinol between parenchymal and nonparenchymal liver cells. In: Knook DL, Wisse E, editors. *Sinusoidal Liver Cells*. Amsterdam: Elsevier; 1982. p. 37-44.

19. Burri BJ, Park J-YK. Compartmental models of vitamin A and  $\beta$ -carotene metabolism in women. *Adv Exp Biol Med* 1998;445:225-37.

20. Parker RS, Swanson JE, You C-S, Edwards AJ, Huang T. Bioavailability of carotenoids in human subjects. *Proc Nutr Soc* 1999;58:155-62.
21. Green MH, Green JB, Berg T, Norum KR, Blomhoff R. Vitamin A metabolism in rat liver: a kinetic model. *Am J Physiol* 1993;264:G509-21.
22. Wang Z, Yin S, Zhao X, Russell RM, Tang G.  $\beta$ -Carotene-vitamin A equivalence in Chinese adults assessed by an isotope dilution technique. *Br J Nutr* 2004;91:121-31.
23. Green MH, Ford JL, Green JB, Berry P, Boddy AV, Oxley A, Lietz G. A retinol isotope dilution equation predicts both group and individual total body vitamin A stores in adults based on data from an early postdosing blood sample. *J Nutr* 2016;146:2137-42.
24. Green MH, Ford JL, Green JB. Retinol isotope dilution is applied during restriction of vitamin A intake to predict individual subject total body vitamin A stores at isotopic equilibrium. *J Nutr* 2016;146:2407-11.
25. Shmarakov IO, Yuen JJ, Blaner WS. Carotenoid metabolism and enzymology. In: Tanumihardjo SA, editor. *Carotenoids and human health*. New York: Humana Press; 2013. p. 29-56.
26. Napoli JL, Race KR. Biogenesis of retinoic acid from  $\beta$ -carotene: differences between the metabolism of  $\beta$ -carotene and retinal. *J Biol Chem* 1988;263:17372-7.
27. Harrison EH, dela Sena C, Eroglu A, Fleshman MK. The formation, occurrence, and function of  $\beta$ -apocarotenoids:  $\beta$ -carotene metabolites that may modulate nuclear receptor signaling. *Am J Clin Nutr* 2012;96:1189S-92S.
28. Lin Y, Dueker SR, Burri BJ, Neidlinger TR, Clifford AJ. Variability of the conversion of  $\beta$ -carotene to vitamin A in women measured by using a double-tracer study design. *Am J Clin Nutr* 2000;71:1545-54.

**TABLE 1** Assigned (known) and predicted values for  $\beta$ -carotene relative bioefficacy (%) for ten subjects<sup>1</sup>

Subject	1	2	3	4	5	6	7	8	9	10
Assigned values										
Total	10	14	18	21	25	30	35	40	45	50
Absorptive	7	10	9	16	20	15	28	30	40	30
Post-absorptive	3	4	9	5	5	15	7	10	5	20
Early	2.4	3	6.75	4.25	2.25	5.25	2.1	2	2.5	2
Late	0.6	1	2.25	0.75	2.75	9.75	4.9	8	2.5	18
Values predicted by RIR										
1 d	8.1	12.9	13.6	19.6	23.0	17.2	29.8	32.7	41.8	31.5
2 d	8.3	11.0	12.0	20.5	22.0	17.1	29.7	32.7	40.8	31.0
4 d	11.2	15.6	23.2	20.6	32.1	40.8	29.3	34.4	41.1	31.0
7 d	10.0	14.2	18.1	22.5	27.3	31.5	47.8	69.2	49.2	62.9
14 d	10.0	14.0	18.1	21.1	25.2	30.4	35.5	40.9	45.1	51.3
21 d	10.0	14.0	18.1	21.1	25.2	30.4	35.4	40.8	45.1	51.4
28 d	10.0	14.0	18.1	21.1	25.2	30.4	35.4	40.8	45.1	51.4
56 d	10.0	14.0	18.1	21.1	25.2	30.4	35.4	40.8	45.1	51.4

## Values predicted by IRM

1 d	10.1	15.6	18.3	20.2	23.3	16.0	27.2	30.1	42.7	29.9
2 d	9.2	13.8	15.6	20.0	23.0	16.6	28.7	31.6	42.1	30.6
4 d	9.2	13.6	15.2	20.1	23.1	17.4	29.0	31.9	41.7	30.7
7 d	9.4	13.9	16.3	20.7	24.5	20.0	30.5	36.8	42.5	36.3
14 d	9.6	13.9	16.8	20.8	24.7	22.1	32.2	38.7	42.9	38.4
21 d	9.7	13.9	17.0	20.8	24.8	23.4	32.6	38.9	43.1	39.5
28 d	9.8	13.9	17.1	20.9	24.8	24.2	32.8	39.1	43.3	40.4
56 d	9.8	14.0	17.3	20.9	24.9	25.8	33.3	39.4	43.7	42.2

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<sup>1</sup> Values are percent  $\beta$ -carotene relative bioefficacy for 10 subjects selected from previously published studies (6, 15). The top section shows values for relative bioefficacy [total, absorptive, and post-absorptive (early and late)] that were assigned to each subject as described in Methods. The middle section shows predictions of the plasma retinol isotope ratio [RIR; Equation 1 (see Methods)] versus time after co-ingestion of an oral dose of stable isotope-labeled  $\beta$ -carotene and a retinyl acetate reference dose; the bottom section shows predictions of the isotope reference method [IRM; Equation 2 (see Methods)] versus time after dosing.

## Figure Legends

- Proposed compartmental models for retinol kinetics in humans following co-ingestion of labeled  $\beta$ -carotene and labeled retinyl acetate. Circles represent compartments, rectangles correspond to delay components, interconnectivities between compartments  $[L(I,J)]$ s are fractional transfer coefficients or the fraction of retinol or  $\beta$ -carotene molar retinol activity equivalents transferred to compartment I from compartment J each day, and delay times  $[DT(I)]$  correspond to the time spent in delay component I. The asterisks represent the site of input of the orally administered tracers; U(1) and U(11) represent dietary vitamin A and  $\beta$ -carotene input, respectively; and the triangles represent the site of sampling (plasma). Panel A shows the six-compartment model adapted from (6) that describes the metabolism of retinyl acetate-derived labeled retinol. Compartments 1 to 4 represent the processing of dietary preformed vitamin A; fractional absorption of retinyl acetate was set at 0.75. Diet-derived vitamin A is taken up by hepatocytes (compartment 4), primarily as retinyl esters, and then retinol is secreted into plasma compartment 5 bound to retinol-binding protein; retinol in the plasma pool exchanges with vitamin A in the extravascular storage pool (compartment 6), which is the site of irreversible loss from the system. Panel B shows the ten-compartment model developed to describe the metabolism of  $\beta$ -carotene-derived retinol during absorptive (intestinal) conversion (unshaded components) as well as early and late post-absorptive (extra-intestinal) conversion (shaded components). Components 11 to 13 represent gastrointestinal transit and absorption of  $\beta$ -carotene; in compartment 11, absorbed  $\beta$ -carotene is partitioned into the fraction eventually converted to retinol (that which is transferred to compartment 12) versus that which is not converted  $[(L0,11)]$ , and in delay component 13, bioefficacy is partitioned into absorptive and post-absorptive components. For the absorptive pathway, retinol (primarily as retinyl esters) derived from absorbed  $\beta$ -carotene is transferred to compartment 14 and processed as described above in compartments 15 and 16. For the post-absorptive pathway, absorbed  $\beta$ -carotene is

transferred from component 13 to compartment 21, representing uptake by hepatic and extrahepatic tissues. For the early post-absorptive pathway (solid-line arrow), some of this  $\beta$ -carotene in compartment 21 is converted in situ to retinol (compartment 24). For the late post-absorptive pathway (dashed-line arrows), the  $\beta$ -carotene passes from compartment 21 through component 22 and compartment 23 before entering compartment 24. Retinol in compartment 24 can either be secreted directly into plasma (compartment 15) or esterified for storage (compartment 16).  $\beta$ C,  $\beta$ -carotene; ROH, retinol; RE, retinyl esters.

2. Model simulations for fraction of the  $\beta$ -carotene dose in various compartments versus time for subject 5. Shown are fraction of dose (as molar retinol activity equivalents) in model compartments (Figure 1B) that were designated as sinks (see Supplemental Methods 1) for total (compartment 12), absorptive (compartment 14), and post-absorptive bioefficacy (compartment 21), as well as for the early (compartment 24 with no input from compartment 23) and late post-absorptive components (compartment 23). Assigned values for bioefficacy (%) are indicated at each curve's plateau.
3. Model-predicted plasma retinol response curves versus time after co-ingestion of labeled  $\beta$ -carotene and labeled retinyl acetate for two subjects. Shown are simulated data for fraction of dose in molar retinol activity equivalents over time for labeled retinol derived from the retinyl acetate reference dose and from the  $\beta$ -carotene dose. Panel A shows simulations for subject 2 (Table 1) who was assigned values of 14% total relative bioefficacy (10% absorptive plus 4% post-absorptive bioconversion) with late post-absorptive conversion set at 2 d; panel B shows the corresponding simulations for subject 8 (40% total, of which 30% was absorptive and 10% post-absorptive); late post-absorptive conversion was set at 3.5 d.
4. Model-predicted plasma retinol response curves versus time after co-ingestion of labeled  $\beta$ -carotene and labeled retinyl acetate for subject 5. Shown are simulated values for fraction of dose in molar retinol activity equivalents over time (shown on a semi-log scale) for labeled retinol derived from the retinyl acetate reference dose and for  $\beta$ -carotene-derived retinol, as

well as retinol formed from absorptive and post-absorptive conversion of  $\beta$ -carotene.

Subject 5 (Table 1) was assigned 25% total relative bioefficacy (20% absorptive plus 5% post-absorptive, with 2.25% early post-absorptive and 2.75% late post-absorptive). The inset shows these simulations for  $\beta$ -carotene-derived retinol on an expanded (linear) scale between 3 h and 10 d.

5.  $\beta$ -Carotene relative bioefficacy calculated by two methods. The plasma retinol isotope ratio (RIR; see Methods, Equation 1) and the isotope reference method (IRM; Equation 2) were used to calculate  $\beta$ -carotene relative bioefficacy over time for subject 7 (Table 1) who was assigned a value of 35% total relative bioefficacy (28% absorptive plus 7% post-absorptive); late post-absorptive conversion was set at 4.5 d post-dosing.
6.  $\beta$ -Carotene relative bioefficacy predicted by two methods for previously-studied adults (6). Values are means  $\pm$  SEM (n=30) for relative bioefficacy calculated at four times after co-ingestion of labeled  $\beta$ -carotene and a labeled retinyl acetate reference dose; values were calculated using the plasma retinol isotope ratio (RIR; see Methods, Equation 1) and the isotope reference method (IRM; Equation 2). Means within each method that have different letters are significantly different ( $P < 0.05$ ) using repeated measures ANOVA.