Intestinal Beta-carotene absorption and cleavage in men: response of Beta-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of Beta-carotene 1-3

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Intestinal \(\beta\)-carotene absorption and cleavage in men: response of \(\beta\)-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of \(\beta\)-carotene\(^1\sim^3\)

Trinette van Vliet, Wil HP Schreurs, and Henk van den Berg

ABSTRACT Postprandial response curves of \(\beta\)-carotene and retinyl esters in a triglyceride-rich lipoprotein (TRL) fraction were evaluated as a potential measure of \(\beta\)-carotene uptake and cleavage. \(\beta\)-Carotene, retinyl ester, and triglyceride concentrations in the TRL fraction (density < 1.006 kg/L) and plasma were measured in 10 men for 8 or 16 h after an oral dose of 15 mg \(\beta\)-carotene. The \(\beta\)-carotene response, unlike the triglyceride and retinyl ester response, can be evaluated in the TRL fraction but not in plasma. Intraindividual variations in the triglyceride-adjusted response of \(\beta\)-carotene and retinyl palmitate in TRL fractions were 23% and 20% and interindividual variations were 42% and 36%, respectively. A low \(\beta\)-carotene response was associated with a high ratio between retinyl palmitate and \(\beta\)-carotene responses \((r = -0.56, P = 0.013)\). In conclusion, the measurement of \(\beta\)-carotene and retinyl esters in the TRL fraction after a dose of \(\beta\)-carotene with a vitamin A–free meal may be an appropriate method to study \(\beta\)-carotene uptake and cleavage.

KEY WORDS \(\beta\)-carotene, retinyl esters, intraindividual variation, interindividual variation, chylomicrons, plasma, triglycerides, single dose, response, second meal

INTRODUCTION

Epidemiological studies indicate that the consumption of foods rich in carotenoids is associated with a reduced risk for certain types of cancer and cardiovascular disease \((1, 2)\). \(\beta\)-Carotene may be preventive as an antioxidant \((3)\) or as a precursor for vitamin A. The factors determining the balance between absorption of intact \(\beta\)-carotene and \(\beta\)-carotene cleavage in humans are still largely unknown. The identification of these factors is not only important in controlling \(\beta\)-carotene antioxidant status, but also in controlling vitamin A status, which is especially relevant in many parts of the developing world where \(\beta\)-carotene is more readily available in the diet than is vitamin A. To identify these factors, a reproducible method to assess intestinal \(\beta\)-carotene absorption and cleavage in humans is needed.

Most of the studies focused on \(\beta\)-carotene absorption and availability used the \(\beta\)-carotene plasma response curve. A wide variability for this response has been reported \((4-11)\). Sometimes the retinyl ester response is also measured \((6, 8, 10, 11)\). However, in most studies the test meal contained vitamin A, it is unclear whether retinyl esters originate from cleaved \(\beta\)-carotene or from vitamin A in the test meal.

Interpretation of plasma \(\beta\)-carotene response curves is hampered by the fact that most of the circulating \(\beta\)-carotene is of endogenous origin and has a long half-life of \(\sim5\) d \((4, 12)\). Chylomicrons, however, contain only absorbed \(\beta\)-carotene and its cleavage products. Therefore, chylomicrons may be a better model for studying intestinal absorption and cleavage of \(\beta\)-carotene. Chylomicron fractions were analyzed after oral dosing in two studies only. These studies consisted of only a limited number of samples, and cleavage products could not be demonstrated \((11)\) or were not measured \((13)\).

A problem with the use of plasma response curves may be nonresponse, recently reported by Johnson and Russell \((11)\) in a relatively high percentage of their subjects. Nonresponders showed no plasma \(\beta\)-carotene response and only a small response in chylomicrons after a large oral dose of 120 mg \(\beta\)-carotene. It was also suggested that the plasma \(\beta\)-carotene response was affected by a delayed release of \(\beta\)-carotene from the enterocyte into the circulation, possibly as a consequence of a second meal as reported by Henderson et al \((6)\).

To evaluate postprandial chylomicron curves as a reliable method to study factors affecting \(\beta\)-carotene intestinal absorption and cleavage in humans, we studied the intra- and interindividual variabilities of this method. Therefore, we determined the response curves of \(\beta\)-carotene, retinyl esters, and triglycerides in a triglyceride-rich lipoprotein (TRL) fraction and plasma of volunteers after a single oral dose of \(\beta\)-carotene on two occasions, 16 d apart. In addition, the possibility of a delayed response was tested by assessing the response after a second meal without \(\beta\)-carotene, 8 h after the \(\beta\)-carotene dose.

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\(\text{2 Hoffmann-La Roche donated the 10% water-soluble \(\beta\)-carotene beadlets used in this study.}\)

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SUBJECTS AND METHODS

Subjects

Twelve healthy, nonsmoking, young men living in the Utrecht, Netherlands, area participated. All volunteers underwent a screening procedure that included a health and lifestyle questionnaire, a physical examination, and a routine blood clinical chemistry profile. Volunteers had no history or biochemical evidence of liver or pancreatic disease, active bowel disease, resection, or abnormal fat metabolism, and did not use medication suspected of interfering with fat-soluble-vitamin absorption. Excessive alcohol consumers (> 40 g/d) were excluded and the volunteers did not use vitamin or carotenoid supplements in the 2 mo before the study.

The study protocol was approved by the Medical Ethical Committee of the TNO Institute and the participants provided written informed consent.

Study design

All participants were instructed to avoid food products with a high vitamin A or provitamin-A-carotenoid content in the week before the study; they were given a list of products to exclude from the diet. During the last 2 d before the study, all participants were supplied with a standardized diet, low in vitamin A (70 µg/d) and β-carotene (11 µg/d), to minimize the presence of β-carotene and dietary retinoids in the intestine, which could interfere with the results.

The study consisted of 2 experimental days, 16 d apart. Day 1 (the first test day) included two consecutive experimental periods of 8 h, the first starting with a meal with β-carotene and the second with the same meal without β-carotene. Ten subjects participated but the results for n-carotene in the TRL content of the meal was 2750 kJ, the meal contained 8.7 mg a-tocopherol, and no vitamin A could be detected (< 3 µg).

The procedure of isolating the TRL fraction from frozen plasma samples was validated by measuring the retinyl palmitate, β-carotene, and triglyceride contents of the TRL fraction isolated from fresh plasma as well as from the same plasma sample after the plasma had been frozen for ≥ 1 d. Plasma samples were collected from nine volunteers after an overnight fast and 5 h after a meal, as in the main study. Only small amounts of β-carotene and retinyl palmitate (both < 8 mmol/L plasma) and triglycerides (< 0.1 mmol/L plasma) could be demonstrated in both the freshly isolated TRL fraction and the TRL fraction from frozen plasma collected after subjects had fasted. For nonfasting plasma the amounts (x ± SEM) of β-carotene, retinyl palmitate, and triglycerides in the TRL fraction isolated from frozen plasma, expressed as a percentage of the amount in the freshly isolated TRL fraction, were 118 ± 6%, 101 ± 4%, and 95 ± 4%, respectively. Amounts in fresh and frozen samples did not differ significantly for any of the compounds analyzed (paired t test).

Analytical methods

Cholesterol and triglyceride concentrations were measured by using commercially available enzymatic colorimetric assays (CHOD-PAP and GPO-PAP, respectively; Boehringer, Mannheim, Germany).

For retinyl ester, β-carotene, and vitamin E analyses 1 mL of the TRL fraction or 1 mL plasma was mixed with 1 mL ethanol (containing 16–32 µmol α-tocopheryl acetate/L as internal standard). After 10 min, 2 mL hexane was added and the sealed tubes were vortexed for 4 min. After centrifugation for 10 min at 3000 × g at 4 °C, the hexane layer was separated and evaporated under nitrogen at room temperature. The residue was dissolved in 0.4 mL HPLC solvent and transferred into brown HPLC injection vials.

Retinyl palmitate, retinyl stearate, β-carotene, and vitamin E were quantified by HPLC, using a modified version of a method previously described (14). Briefly, a Superspher 100 RP-18 column (Merck, Darmstadt, Germany) was used. The mobile phase consisted of acetonitrile:methylene chloride: methanol (70:15:10, by vol) and the flow rate was 1 mL/min. Two absorbance detectors in series were used, one switching from 292 to 445 nm for detection of tocopherols and β-carotene, respectively, and the other switching from 292 to 325 nm for detection of tocopherols and retinyl esters, respectively. Limits of detection for the TRL fraction were 1.6 and 3.7 nmol/L plasma for β-carotene and retinyl palmitate, respec-
respectively, and for plasma these limits were 3.0 and 5.5 nmol/L plasma.

Standards of \( \alpha \)-tocopherol and \( \alpha \)-tocopheryl acetate were obtained from Sigma (St Louis), \( \beta \)-carotene from Merck, and retinyl palmitate from Fluka (Buchs, Switzerland). Retinyl stearate containing small amounts of palmitate was a gift from Hoffmann-La Roche (Basel, Switzerland). The stearate content was determined by correcting spectrophotometric readings of a stearate solution for the HPLC-determined palmitate content, using an \( E_{1\%}^{1\%} \) (the absorbance of a 1% solution) of 975 for palmitate and 940 for stearate, both in ethanol at 325 nm (15). Retinyl palmitate is the most abundant retinyl ester and the amount of stearate was a fixed percentage of palmitate, independent of subject or time point (on average 34%). Because retinyl stearate was difficult to quantitate at early and late time points, only the results of retinyl palmitate are given. The sum of retinyl palmitate and retinyl stearate, mentioned as retinyl esters, is used for estimation of \( \beta \)-carotene conversion and absorption.

Statistics

Results were expressed as mean ± SEM. Results for days 1 and 16 were compared by using the paired Student’s \( t \) test. Pearson’s correlation coefficients were used to assess the relation between results for day 1 and those for day 16, and between different responses. Two-sided \( P \) values < 0.05 were considered statistically significant. Estimates of the intra- and interindividual SDs were calculated from the pooled data by one-way analysis of variance. Changes in the ratio of retinyl esters to \( \beta \)-carotene in TRLs with time were evaluated by fitting orthogonal polynomials for all subjects. A Student’s \( t \) test was used to test whether the mean quadratic coefficient of the polynomials was significantly greater than zero, indicating the presence of a U-shaped component.

RESULTS

Characteristics of the subjects are presented in Table 1. Normal ranges from the laboratory for fasting plasma concentrations established with apparently healthy blood donors aged 16–64 y were 15–43 \( \mu \)mol/L for vitamin E and 100–800 nmol/L for \( \beta \)-carotene. Generally reported cutoff points of < 2.3 and 6.5 mmol/L were adapted for triglycerides and cholesterol, respectively. For all volunteers the vitamin E and cholesterol concentrations were within the normal range. One volunteer was accepted with a screening triglyceride concentration of 2.6 mmol/L (< 2.3 mmol/L on both test days). The \( \beta \)-carotene concentrations of four subjects were below the lower limit of the normal range, probably because the subjects consumed a diet low in \( \beta \)-carotene for 1 wk before the test days. No differences were observed between the 10 experimental subjects and the 2 control subjects.

Response in TRLs

For all subjects given \( \beta \)-carotene, there was a positive response of triglycerides, \( \beta \)-carotene, and retinyl palmitate in the TRL fraction, although the variation in response was large. Mean curves for triglycerides, \( \beta \)-carotene, and retinyl palmitate in the TRL fraction on days 1 and 16 are given in Figure 1. The mean triglyceride curves peaked early at \( \approx \)2 h whereas a second peak was seen 5–6 h after the first meal curves for \( \beta \)-carotene and retinyl palmitate peaked at 5–6 h; the \( \beta \)-carotene peak was symmetrical whereas the retinyl palmitate peak seemed skewed to the right.

### TABLE 1

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>22.2 ± 0.3 (20–24)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.7 ± 0.6 (19.4–24.5)</td>
</tr>
<tr>
<td>Fasting plasma concentrations</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.8 ± 0.2 (3.6–5.7)</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.3 ± 0.2 (0.7–2.6)</td>
</tr>
<tr>
<td>( \beta )-Carotene (mmol/L)</td>
<td>159 ± 27 (47–447)</td>
</tr>
<tr>
<td>Retinyl palmitate (nmol/L)</td>
<td>14.7 ± 3.1 (4.6–59)</td>
</tr>
<tr>
<td>Vitamin E (( \mu )mol/L)</td>
<td>23.4 ± 1.5 (15–35)</td>
</tr>
</tbody>
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\( ^{1} \) ± SEM; range in parentheses. \( n = 12 \).

FIGURE 1. Response of triglyceride, \( \beta \)-carotene, and retinyl palmitate in the triglyceride-rich lipoprotein (TRL) fraction after a single oral dose of 15 mg \( \beta \)-carotene with a test meal and a second meal (\( \beta \)-carotene–free) 8 h later. ●, day 1; †, day 16. ± SEM; \( n = 10 \) (\( n = 8 \) for \( \beta \)-carotene on day 1).
For quantitative comparisons between and within subjects, and to estimate β-carotene absorption, the areas under the concentration time curves (AUCs) were calculated by using trapezoidal approximation after subtracting baseline concentrations. The results are presented in Table 2. Although the triglyceride response tended to be higher on day 16 than on day 1, no significant differences were found between the experimental days (paired t test). The control subjects, who only participated on day 16, showed a triglyceride response comparable with that of the subjects (1.75 and 3.15 mmol·h/L), whereas one control subject showed no retinyl palmitate and β-carotene responses and the other showed very low responses, never exceeding 18% of the mean values for subjects on day 16.

The second meal on day 1 resulted in a triglyceride response not different from the response to the first meal; the AUC at 0–8 h was 1.93 ± 0.47 mmol·h/L and the AUC at 8–16 h was 1.73 ± 0.27 mmol·h/L. For both β-carotene and retinyl palmitate the mean curves showed no increase in response to the second meal. Seven subjects displayed no response to the second meal, whereas three subjects showed a response with an AUC 73%, 29%, and 13% (mean of the β-carotene and the retinyl palmitate response) of the AUC after the first meal.

**Plasma response**

Mean plasma curves for triglycerides, β-carotene, and retinyl palmitate on days 1 and 16 are shown in Figure 2. The curves for triglycerides and retinyl palmitate were quite similar to the curves of the TRL fraction because baseline plasma concentrations were low. Fasting plasma β-carotene concentrations were much higher; therefore, the β-carotene response in plasma was less clear. An increase in the β-carotene concentration until 16 h was seen in plasma but not in the TRL fraction. The increase in plasma may have been due to resecretion of β-carotene taken up by the liver. For retinyl palmitate the AUC of plasma was calculated (Table 2).

**Intra- and interindividual variation**

Intra- and interindividual variabilities for the AUCs of triglycerides, β-carotene, and retinyl palmitate in TRLs and for fasting plasma concentrations are presented in Table 3, together with the ratios of intra- to interindividual variation. Both the β-carotene and retinyl palmitate responses in TRLs were strongly correlated with the triglyceride response (both days together, r = 0.81 and 0.92, respectively; P < 0.0001). Because the triglyceride response varied widely between the 2 experimental days, the responses of β-carotene and retinyl palmitate were adjusted for the triglyceride response. As shown, adjustment reduced intraindividual and to a lesser extent interindividual variations, and improved the ratio of intra- to interindividual variability.

**Ratio of retinyl esters to β-carotene**

To get an impression of the intestinal β-carotene cleavage activity, the ratio of AUCs for retinyl esters to AUCs for β-carotene in the TRL fraction was calculated. This ratio varied widely between subjects from 1.07 to 4.85 (x; 2.92 ± 0.41 for day 1 and 2.43 ± 0.30 for day 16), but was reproducible between both days (r = 0.87, P = 0.0021).

Efficient conversion, defined as a high ratio between retinyl esters and β-carotene, was related to a low AUC for β-carotene, as is shown in Figure 3 (r = −0.56, P = 0.013). No relation between conversion and the sum of the retinyl ester and β-carotene response adjusted for triglyceride response could be demonstrated.

As mentioned before, the shapes of the β-carotene and retinyl palmitate curves were not identical. To further study this difference the mean ratio of retinyl esters to β-carotene at all time points after the meal with β-carotene is shown in Figure 4. After a decline during the first hours after the meal, a gradual increase was seen. The mean quadratic component of the fitted individual polynomials was significantly greater than zero (P < 0.002), indicating that the curves do contain a U-shaped component.

**DISCUSSION**

In this study we measured the individual responses of β-carotene and retinyl esters in TRL fractions and plasma after a single oral dose of β-carotene. The data show that the β-carotene response, unlike the response of triglyceride and retinyl ester, after a single oral dose of β-carotene can be evaluated in TRLs but not in plasma.

Reported β-carotene plasma responses from other studies are highly variable (4–11), because of differences in the dose and administration of β-carotene (5, 6, 13), test meal composition (7, 9, 16), meal pattern during the test (6), and subject characteristics (8, 16, 17). Because of this variability, quantitative comparison with our results is not useful.

The use of TRL response curves as a measure for β-carotene uptake and cleavage is based on the assumption that the TRL fraction contains mainly intestinally derived lipoproteins (chy-
In our study the recovery of intestinally palmitate in the TRL fraction (21-23). Mean absorption (β-carotene plus retinyl esters) was 11% assuming central cleavage, ie, 2 molecules of retinyl esters formed per molecule of β-carotene, or 17% assuming eccentric cleavage, ie, 1 molecule of retinyl ester formed per molecule of β-carotene. We realize that this is a rough estimate, which can only be made on a group level, but the results are in reasonable agreement with the 9–17% absorption (with one extreme of 52%) found in lymph cannulation studies in humans (29, 30).

For comparative studies on factors affecting β-carotene absorption and cleavage, a reproducible measure, ie, a relatively low intraindividual variability compared with interindividual variability, is required. As shown in Table 3, the best results for β-carotene and retinyl palmitate in TRLs were obtained after adjustment for the triglyceride response. The large intraindividual variability in the triglyceride response in our study (62%) compared with reported values of 19% (31) and 6.4% (23) could be explained by differences in fasting plasma triglyceride concentrations. The fasting plasma triglyceride concentration is reported to be an important determinant of the triglyceride response (32). Adjustment of our triglyceride response by dividing the AUC for triglyceride by the fasting plasma triglyceride concentration resulted in AUCs of 1.83 ± 0.34 and 2.28 ± 0.29 mmol·h/L for days 1 and 16, respectively; a reduction of the intraindividual variability from 62% to 28%; and a reduction of the ratio from 1.58 to 0.68.

SDs in fasting plasma concentrations of β-carotene, retinyl palmitate, and triglyceride found in our study (Table 3) were in good agreement with reported values (33, 34).

Limitations of the method, such as nonresponse or delayed response, seemed unimportant in our study. All subjects that received β-carotene showed a response and only one subject showed an appreciable response of β-carotene and retinyl ester to the second meal compared with the first meal. The relatively low dose of β-carotene given in combination with a fair amount of fat resulted in maximal absorption with the first meal.

To further evaluate our method, β-carotene conversion was estimated by using the ratios between the AUCs for retinyl esters and β-carotene in the TRLs. Assuming a similar clearance for retinyl esters and β-carotene, the ratios found correspond with 35–71% of β-carotene conversion when only cen-
tral cleavage occurred and 52–83% when only eccentric cleavage occurred, ie, there was only one molecule of retinyl ester formed per molecule β-carotene. These percentages are similar to those (67–94%) calculated from the results of the lymph cannulation studies mentioned before (29, 30).

The assumed similarity of retinyl palmitate and β-carotene clearance was supported by the fact that maximum concentrations of β-carotene and retinyl palmitate were reached at the same time. Furthermore, calculated half-lives for both compounds in the TRLs over the period from the maximum concentration to 8 h after the dose were not different (paired t test) and correlated well (both days together, \( r = 0.68, P = 0.0015 \)).

The balance between cleavage and absorption of intact β-carotene differed between subjects. A low β-carotene response seemed to be related to a high retinyl ester response (Figure 3), but not to a low total response (sum of β-carotene and retinyl ester responses). The absence of a β-carotene response may be explained by very efficient conversion, although this was not seen in our study.

Intestinal metabolism of β-carotene may not be a continuous process (Figure 4). The decline of the ratio in the first hours after the β-carotene dose can possibly be explained by product inhibition or by saturation of the enzyme. The establishment of a new equilibrium may explain the increase of the ratio after 4 h. Some form of regulation of the enzyme activity is expected because a high β-carotene intake does not lead to vitamin A intoxication (35). Another possible explanation for the decline is the faster incorporation of retinyl esters into chylomicrons, compared with β-carotene. As described by Ong (36), retinal formed from intestinal cleavage of β-carotene is probably bound to cellular retinoid-binding protein II and efficiently converted to retinol and subsequently to retinyl esters and incorporated in chylomicrons. The mechanism of β-carotene transport through the cell is not known, but may be less efficient. This theory, however, cannot explain the increase of the ratio from 4 h on.

In summary, our study shows that the use of response curves of β-carotene and retinyl palmitate in TRLs after a single oral dose of β-carotene is an appropriate method to evaluate intestinal absorption and cleavage of β-carotene in humans.
reproducible data were obtained after adjustment of the \(\beta\)-carotene and retinyl palmitate responses (expressed as the AUC) for the triglyceride response. This method enables exploratory studies on factors affecting \(\beta\)-carotene absorption and cleavage. The ratio of the response of retinyl esters to \(\beta\)-carotene may be a good indicator for intestinal \(\beta\)-carotene conversion.

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