Intestinal absorption of \( \beta \)-carotene, lycopene and lutein in men and women following a standard meal: response curves in the triacylglycerol-rich lipoprotein fraction

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A high intake of fruit and vegetables is believed to be protective against heart disease and cancer. \( \beta \)-Carotene has been closely examined for evidence of these protective properties but evidence is still conflicting and there are many other carotenoids in plant foods which deserve attention. This paper reports studies on the concentrations of lutein and lycopene in the triacylglycerol-rich lipoprotein (TRL) fraction of plasma in comparison with \( \beta \)-carotene following a large dose of the respective carotenoids fed with a standard meal after an overnight fast. \( \beta \)-Carotene (40 mg) was given to twelve volunteers (six men and six women) and six of the same volunteers (three men and three women) also received 31.2 mg lutein or 38 mg lycopene. Plasma was collected at hourly intervals for 8 h and the TRL fraction was separated and subsequently analysed for the respective carotenoids and retinyl palmitate in the case of \( \beta \)-carotene. Intestinal uptake of the three carotenoids was estimated using the ‘area under the curve’ method and apparent absorption was calculated from these results. The response curves in the TRL fraction for \( \beta \)-carotene and retinyl palmitate occurred maximally over the fourth to fifth hour postprandially. There was a correlation between the TRL concentrations of \( \beta \)-carotene and retinyl palmitate (males \( r \) 0.62, \( P < 0.001 \); females \( r \) 0.52, \( P < 0.001 \)) and there was no significant difference between men and women either in the total amount of \( \beta \)-carotene appearing in the TRL fraction or in the amount converted to retinol. On estimation, approximately 1.4 mg of the 40 mg \( \beta \)-carotene dose was absorbed and this was not significantly different from the amount of lycopene (1.0 mg) but significantly different (\( P = 0.05 \)) from the amount of lutein (0.8 mg) absorbed, after correction for the smaller doses administered. There was approximately a twofold difference between subjects in the uptake of \( \beta \)-carotene into the TRL fraction, a two- to threefold variation in lycopene and a two- to threefold variation in lutein. Despite these inter-subject differences, in three volunteers between whom there was a threefold difference in \( \beta \)-carotene in the TRL fraction and a twofold difference in retinol formation, repeat experiments with \( \beta \)-carotene 4 months later found differences of only 3-6\% in the TRL \( \beta \)-carotene content and 4-9\% for the TRL retinol formed. In conclusion, large inter-subject variation in TRL carotene uptake precluded any differences between sexes but surprising intra-subject consistency was observed in TRL \( \beta \)-carotene uptake of three subjects.

Carotenoids: \( \beta \)-Carotene: Lutein: Lycopene

There is considerable interest in phytoprotective substances since a high dietary intake of fruit and vegetables has been associated with a lower risk of both heart disease and cancer in many epidemiological studies (Hennekens, 1986; Committee on Diet and Health, 1989; Hertog et al. 1993). \( \beta \)-Carotene has been the focus of much of this attention (Gey, 1993), as its dietary importance as a source of vitamin A has been long established. However, if it is the antioxidant properties which are of importance in reducing the risk of disease (Gey, 1986) then there are many other carotenoids in our diet with such properties (Terao, 1989); for example, lutein, which is found specifically in the macula lutea (Handelman et al. 1988; Bone et al. 1993), may be of particular biological relevance.

Most studies investigating the absorption of carotenoids have focused on \( \beta \)-carotene (Krinsky et al. 1958; Goodman et al. 1966; Dimitrov et al. 1986; Olson, 1989; Sugerman et al. 1991; Johnson et al. 1992; Van Vliet & Van den Berg, 1997).

Abbreviations: AUC, area under the curve; TRL, triacylglycerol-rich lipoproteins.

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More recently two groups have specifically examined the uptake of lutein from the gut into the plasma (Kostic et al. 1995) or into chylomicrons as compared with the all \textit{trans} \(\beta\)-carotene (Gartner et al. 1996) but most other authors have examined the absorption and/or interactions of mixtures of carotenoids (Brown et al. 1989; Micozzi et al. 1992; Carughi & Hooper, 1994; Bierer et al. 1995; Gartner et al. 1996). Even so, knowledge of the specific factors affecting absorption, transport and metabolism of the different carotenoids is still in its infancy and even for \(\beta\)-carotene, there is no consensus on the efficiency of the intestinal absorption, and the mechanism of its cleavage to retinol is still actively debated (Olson, 1989; Wang et al. 1991).

After absorption of \(\beta\)-carotene in the small intestine, most \(\beta\)-carotene is believed to be converted to vitamin A in the intestinal mucosa, and only a small part may be transported intact into the blood (Goodman et al. 1966; Bloomstrand & Werner, 1967). In the Western diet, it has been suggested that 25–50\% of vitamin A is provided by pro-vitamin-A carotenoids, in particular \(\beta\)-carotene (Olson, 1989). Most studies of \(\beta\)-carotene absorption have used plasma response curves to assess the response to dietary intake (Dimitrov et al. 1986; Brown et al. 1989; Henderson et al. 1989; Mobarhan et al. 1990; Sugerman et al. 1991; Johnson & Russell, 1992; Rock & Swendsen, 1992; Prince & Frisoli, 1993) and widely variable results have been shown by this method. However, the triacylglycerol-rich lipoprotein (TRL; i.e. chylomicrons and chylomicron remnants) fraction of plasma is a better vehicle to assess recently absorbed \(\beta\)-carotene and any cleavage products. In contrast, plasma may contain \(\beta\)-carotene taken up from many meals, and it could take 4–12\,d for the plasma \(\beta\)-carotene level to return to baseline following a single oral dose (Dimitrov et al. 1986).

The use of the serum response curve to measure the absorption of lutein following an oral dose of 0.5\,\mu\text{mol} \(\beta\)-carotene/kg body weight (approximately 20\,mg), and its interaction with \(\beta\)-carotene, have recently been reported by Kostic et al. (1995). What is very obvious from this work, and that of others (Micozzi et al. 1992; Van Vliet & Van den Berg, 1995) is that individual responses can differ markedly and more work is needed in different scenarios to determine the relative importance of different factors in absorption.

This is an observational study in men and women to investigate the appearance of carotenoids and retinyl esters in the TRL fraction after a single oral dose of one of the three carotenoids administered with a standard meal, namely \(\beta\)-carotene, lycopene and lutein. In addition, reproducibility of the TRL response curves for \(\beta\)-carotene in the TRL fraction was studied in three subjects.

Subjects and methods

Subjects

Twelve volunteers, six males and six females aged 20–25 years were recruited for this study. All subjects underwent a screening procedure to ensure that only subjects with a BMI of 20–25\,kg/m\(^2\), a fasting plasma cholesterol concentration of <6.0\,mmol/l and normal triacylglycerol values (0.84–1.94\,mmol/l) were included. In addition, subjects were excluded if they smoked, were pregnant, or had diseases which may interfere with absorption e.g. coeliac or Crohn’s disease, and other chronic diseases such as diabetes. Ethical approval was obtained from the ethical committee at the University of Ulster, Coleraine, UK.

All twelve volunteers took part in the \(\beta\)-carotene feeding experiment and in addition six of the subjects (three men and three women) also took part in the lutein and lycopene-feeding studies, with three subjects (two male and one female) taking part in the \(\beta\)-carotene-feeding experiment on a second occasion (4 months apart).

Protocol

All participants were instructed to avoid foods rich in carotenoids and vitamin A 1\,wk before the study. Such foods included dark green vegetables, tomatoes, tomato products and carrots and subjects were asked to fast for 12\,h overnight immediately before the study. The study day was of 9\,h duration. Baseline blood was taken at 0\,h (approximately 08.00\,hours) and a cannula (Adyste, Becton Dickinson, Spain) was inserted into an antecubital vein of the volunteer. The encapsulated carotenoids were administered within the standard meal which consisted of Rice Krispies (Kelloggs), white bread (Allinsons), egg-white, cheese and bacon. The last three items were prepared as an omelette using 4\,g added (local) butter. The standard meal provided 4.0\,MJ (965\,kcal), 43\,g fat, 52\,g protein, 96\,g carbohydrate and <300\,\mu\text{g} carotenoids. The capsules were administered with the standard meal. Postprandial blood samples (10\,ml) were collected into lithium heparin tubes hourly for 8\,h in most experiments. In addition \(\beta\)-carotene absorption was re-examined in three of the subjects (one female and two males) over a 12\,h postprandial fast. During the study period only water was permitted.

Capsules

\(\beta\)-Carotene and lutein were obtained from Quest (Unilever, Vlardinghen, The Netherlands) and Makhleshim (Beer Sheva, Israel) supplied the lycopene capsules. All capsules were packed by Scherer (St Petersburg, FL, USA). The \(\beta\)-carotene capsules contained carotenes extracted from palm oil and each capsule contained 10\,mg \(\beta\)-carotene, of which approximately 20\% was of \textit{cis} form. Lutein was obtained from marigold flowers and each capsule contained 7.8\,mg lutein esters. Lycopene was obtained from tomatoes and each capsule contained 9.5\,mg. Four capsules were given with the standard meal.

Isolation of plasma and triacylglycerol-rich lipoproteins

Analyses of carotenoids in TRL and plasma were performed on the day of blood sampling. Plasma was collected after centrifugation at 2000\,g for 10\,min at 4\,\degree C. For isolation of the TRL, a 2.5\,ml portion of plasma was transferred into a 5\,ml polycarbonate tube (Nalgene,
Rochester, New York, USA) and overlaid with 2 ml NaCl solution (density 1.006 kg/l). The samples were centrifuged for 30 min at 140,000g in a fixed-head rotor (70.1 Ti Beckman, XL-70, California, USA). The TRL-containing the supernatant fraction was then removed (0.5 ml) from each tube and placed in the dark until further analysis (1 h).

**Analytical methods**

Cholesterol and triacylglycerols were measured on a Cobas Fara centrifugal analyser (Roche Products Ltd, Welwyn Garden City, Herts, UK) using commercially available kits (Randox, Crumlin, Co. Antrim, UK).

Retinol, retinyl esters and carotenoids were analysed by liquid chromatography (Thurnham et al. 1988). Plasma (0-1 ml) or TRL (0.4 ml) was extracted into heptane, evaporated to dryness under N2 and reconstituted in 0.1 ml mobile phase containing 0.1 g butylated hydroxytoluene/l. A single extraction step was used for plasma whereas the TRL fraction was extracted twice, and the extracts pooled before evaporation and reconstitution in mobile phase. The mobile phase consisted of acetonitrile–methanol–dichloromethane (50:50:6, by vol.) and the flow rate was 1 ml/min. Injected samples (50 μl) were separated in mobile phase on a 100 × 4-6 mm cartridge column of 3 μm particles of Spherisorb ODS-2 (PhaseSep Inc., Deeside, Clwyd, UK). Pooled plasma samples were run with every batch of samples as in-house controls. These samples have defined ranges which are used to check efficiency of extraction and HPLC sampling. In addition, the intra-batch CV of a pooled sample varied between 5 and 10% for the carotenoids and retinol measured in this study. The HPLC system comprised a Waters 501 pump, a Wisp 710B autosampler and a 4-channel detector (Millipore Waters, Harrow, Middlesex, UK), carotenoids were measured at 450 nm, while retinol and retinyl esters were measured at 325 nm. The Maxima 820 chromatographic data-handling system (Millipore Waters) was used to collect and integrate the data. Concentrations of analytes (μmol/l) were calculated using response factors obtained using the appropriate extinction coefficients.

**Statistical analysis**

The area under the curve (AUC) method was used to quantify the absorption of the carotenoids and the trapezoidal approximation (Altman, 1992) was used after subtracting baseline concentrations. To estimate the mean amount of a carotenoid absorbed, the AUC calculated from a standard oral dose of the carotenoid was divided by an estimated AUC after a hypothetical intravenous dose of the same size. The formula for calculating fractional absorption of the oral dose is as follows:

\[
\text{fractional absorption} = \frac{\text{calculated oral AUC} \times \text{mass} \times \text{plasma volume}}{\text{oral dose} \times \ln 2}.
\]

assuming \( t_b \) of chylomicrons is 0.192 h (i.e. 11.5 mins) and that carotenoid clearance follows the same kinetics (Cortner et al. 1987; Redgrave et al. 1993) and plasma volume (ml) is (927 + (31.47 × mean body weight (kg))) (Grundy & Mok, 1976). Total oral β-carotene absorption AUC (nmol/l/h) was assessed by summing the figures for β-carotene AUC and retinyl palmitate AUC. That is, it was assumed that 1 mol of β-carotene was converted to 1 mol retinyl palmitate which is assumed to have the same \( t_b \) (eccentric cleavage hypothesis) (Glover, 1960). Mass is the molecular mass of the carotene studied, and the following were used (Da): 536-9 for β-carotene, 536-9 for lycopene, and 568-9 for lutein.

Results were expressed as the means and standard deviations, except where the data were skewed when medians and interquartile ranges were used. Parametric and non-parametric tests, as appropriate, were used to compare the results obtained for males and females. \( P \) values < 0.05 were considered statistically significant. The one-way ANOVA was used to calculate the intra- and inter-individual variation between treatment groups. Due to large inter-individual variations, attempts were made to adjust the data using the triacylglycerol response in the respective TRL fractions but this process did not reduce the CV so it was abandoned.

**Results**

Subject characteristics are presented in Table 1. Plasma cholesterol and triacylglycerol values of all individuals were within the acceptable ranges. Mean fasting β-carotene, lutein and lycopene levels were 494, 341 and 898 nmol/l respectively. Lycopene levels were higher than those of the other carotenoids which is similar to the concentrations for lycopene of 892 (SD 336) nmol/l reported by Micozzi et al. (1992), in a college-aged population similar to our own study group.

In all subjects given the 40 mg dose of β-carotene and the standard meal, there was an increase in TRL although the variation between subjects was large. The median response curves for β-carotene treatment for women and men separately are indicated by the β-carotene and retinyl palmitate content of the TRL fraction (Fig. 1), and maximum triacylglycerol concentrations peaked in the third hour in both sexes (results not shown). For β-carotene, the maximal peak in the TRL fraction occurred between the third and fifth hours in both sexes and for retinyl palmitate maximal peak occurred during the third or fourth hour (Fig. 1). Response curves (i.e. AUC measurements for all subjects) for β-carotene and retinyl palmitate in the TRL fraction were significantly correlated in both males (\( r = 0.62, P < 0.001 \)) and females (\( r = 0.52, P < 0.001 \)) (Spearman correlation). In an attempt to decrease inter-individual variation, the responses of β-carotene and retinyl palmitate were adjusted for the triacylglycerol response. However, using the CV (%) as a measure of variability suggested that the correction makes no difference to inter-individual variation for β-carotene or total β-carotene and increased the variation in the retinol results from 40 to 70% (Table 2).
Table 1. Volunteers, characteristics and baseline measurements

<table>
<thead>
<tr>
<th>Volunteer no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Weight (kg)</th>
<th>Carotenoids studies*</th>
<th>Total plasma cholesterol (mmol/l)</th>
<th>Plasma triacylglycerol (mmol/l)</th>
<th>Plasma glucose (mmol/l)</th>
<th>Plasma β-carotene (nmol/l)</th>
<th>Plasma lutein (nmol/l)</th>
<th>Plasma lycopene (nmol/l)</th>
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<td>1,2,3 (2)</td>
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<td>4.1</td>
<td>154</td>
<td>340</td>
<td>767</td>
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</table>

Median: 4.50 1.09 4.65 345 404 563

* 1, participation in β-carotene study (40 mg); 2, participation in lutein study (31.2 mg); 3, participation in lycopene study (38 mg); (2), participation in two β-carotene experiments (one 8 h and one 12 h experiment).

Fig. 1. Concentrations of β-carotene and retinol palmitate in postprandial triacylglycerol-rich lipoproteins (TRL) of (a) female (n=6) and (b) male (n=6) subjects receiving 40 mg β-carotene in a standard meal containing 43 g fat. Subjects subsequently fasted for 8 h. TRL were obtained by centrifuging 2.5 ml plasma overlaid with 2.0 ml NaCl (1.006 kg/l) at 140,000 g for 30 min. TRL were harvested at each time point for analysis of β-carotene and retinol palmitate by HPLC and cholesterol and triacylglycerols by enzymic kits. Data points represent individual concentrations in the TRL after substraction of baseline values (0 h) and the median concentration at all time points is shown by a line.
The AUC was used to estimate the amount of carotene absorbed. AUC of triacylglycerols, β-carotene and retinyl palmitate for the twelve subjects receiving 40 mg β-carotene are shown in Table 2. The median total AUC calculated for β-carotene (β-carotene + retinyl palmitate) were 217 (interquartile range 132–312) and 168 (interquartile range 106–226) nmol.h/l in males and females respectively. Likewise for retinyl palmitate alone, the median AUC was slightly greater in males 101 (interquartile range 70–120) than in females 75 (interquartile range 38–87) nmol.h/l (NS, Mann–Whitney). To estimate the amount of β-carotene absorbed from the 40 mg capsule the sum of the AUC for β-carotene and retinyl esters in TRL was divided by the estimated mean AUC for β-carotene after a hypothetical intravenous dose (calculation shown on p. 151), where the mean plasma volumes were 3.441 (SD 0.11) and 2.963 (SD 0.23) litres in males and females respectively. Intestinal absorption values estimated from the mean AUC were found to be 1.54 and 0.98 mg from the 40 mg capsule or approximately 3.9 and 2.5 % absorbed, in males and females respectively. Approximately 42 % of the total β-carotene absorbed was converted to retinyl palmitate and the rest was absorbed as β-carotene. In total over the 8 h, median retinyl palmitate concentration represented 46 % of the β-carotene absorbed in males (n 6) and 34 % in females (n 6). In order to eliminate the possibility that a postprandial response would occur in carotenoids after ingestion of the standard meal alone, blank feeding studies were carried out on subjects (n 11, six male, five female) under the same strict experimental conditions. Negligible or no response was observed in the TRL fraction for any of the carotenoids (results not shown).

In Table 3, the AUC data for lutein and lycopene are shown for six of the subjects in whom information was also available on AUC values after supplementation with β-carotene. The data are shown as both the absolute amounts (nmol.h/l) as well as the amount expressed as a percentage of the β-carotene value after correction for the lower intakes of lutein (31.2 mg) and lycopene (38 mg). There was a 2-fold variation in the amount of β-carotene absorbed between subjects, a 2–3-fold variation in lycopene and a 2–3-fold variation in the absorption of lutein. The concentration of total β-carotene (sum of β-carotene and retinyl palmitate) in the TRL fraction, was greater than the

![Graph](https://www.cambridge.org/core/core.png)
concentration of the other carotenoids in all subjects with the exception of one subject (no. 7) who had a high lycopene value. Intestinal uptake of total β-carotene was significantly higher than that of lutein (P < 0.05, paired t test) and although the AUC for lycopene was also lower than total AUC for β-carotene the difference was not significant. After correction for the lower doses of lutein and lycopene administered to the β-carotene standard dose of 40 mg (Table 3), the significant difference (P < 0.05) between the β-carotene and lutein AUC remained.

Figs. 2 and 3 show the individual plasma concentrations of β-carotene and retinyl palmitate respectively in three volunteers who agreed to consume the 40 mg β-carotene on two separate occasions, the second 4 months after the first. The only difference between the two experiments was that on the second of these occasions, measurements of chylomicron components were made over 12 h on the fasting subjects instead of 8 h. Fig. 2 shows AUC data for total β-carotene on the two occasions for the three subjects separately. For subjects 2 and 7, peak responses appeared in the TRL fraction at the same time (third hour) on each occasion, however the peak response for subject 1 varied between the third and sixth hours postprandially. During the period from 7–12 h post-meal, the concentration of β-carotene in all three cases fell to a low plateau level. Fig. 3

Table 2. Areas under the curves (AUC: nmol h/l) for β-carotene and retinyl palmitate in the triacylglycerol-rich-lipoprotein fraction of plasma from subjects receiving 40 mg encapsulated β-carotene with a standard meal* (individual values, uncorrected and corrected for triacylglycerols)

<table>
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<tr>
<th>Subject</th>
<th>Sex</th>
<th>β-Carotene</th>
<th>Retinyl palmitate</th>
<th>Total</th>
<th>Retinyl palmitate (% total β-carotene)</th>
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<td>(corrected)†</td>
<td>(corrected)†</td>
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</tr>
</tbody>
</table>

Median: 113.2; Mean (SD): 127.6 (78.2); Median (50%) = 113.2; Mean (50%) = 137.1 (85.3); Median (25–75%) = 82.5 (32.9–198.0 (93.6)); Median (90%) = 216.1 (114.2-219.5 (174.9)).

Table 3. Areas under the curves (nmol h/l) for β-carotene, lutein and lycopene in the triacylglycerol-rich-lipoprotein fraction of plasma from subjects receiving these carotenoids in encapsulated form with a standard meal* (individual values, before and after correction for the dose)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Lutein†</th>
<th>Lutein (%)‡</th>
<th>Lycopene†</th>
<th>Lycopene (%)§</th>
<th>β-Carotene</th>
<th>Retinyl palmitate</th>
<th>Total β-carotene‖</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>85</td>
<td>28</td>
<td>120</td>
<td>40</td>
<td>286</td>
<td>97</td>
<td>382</td>
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<tr>
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<td>M</td>
<td>56</td>
<td>26</td>
<td>132</td>
<td>51</td>
<td>207</td>
<td>67</td>
<td>274</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
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<td>53</td>
<td>108</td>
<td>65</td>
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<td>140</td>
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<tr>
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<td>F</td>
<td>139</td>
<td>68</td>
<td>263</td>
<td>136</td>
<td>99</td>
<td>104</td>
<td>203</td>
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<td>F</td>
<td>98</td>
<td>72</td>
<td>145</td>
<td>88</td>
<td>128</td>
<td>46</td>
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<tr>
<td>10</td>
<td>F</td>
<td>98</td>
<td>34</td>
<td>116</td>
<td>42</td>
<td>213</td>
<td>77</td>
<td>290</td>
</tr>
</tbody>
</table>

Median: 91; Mean (SD): 126 (58); Median (50%) = 126; Mean (50%) = 167 (87); Median (90%) = 167 (87).
Intestinal absorption of carotenoids in man

Fig. 2. Concentrations of \( \beta \)-carotene in postprandial triacylglycerol-rich lipoproteins (TRL) from three individual subjects who received 40 mg encapsulated \( \beta \)-carotene with a standard meal on two separate occasions approximately 4 months apart. (a), Subject no. 1, male; (b), subject no. 2, male, (c), subject no. 7, female. Blood was collected at hourly intervals for 8 h on the first occasion and 12 h on the second occasion, while the subjects fasted. Curves shown are smoothed plots through the specific points at the times shown.

shows similar AUC data for the retinyl palmitate in the chylomicron fractions for the three subjects individually on the two separate occasions. The peak response was similar for each subject, on both occasions peaking at 4, 3, and 2 h for subjects 1, 2, and 7 respectively. Figs. 2 and 3 provide evidence for the intra-subject reproducibility of the response to the \( \beta \)-carotene supplement. The percentage difference in the amount of total \( \beta \)-carotene in the TRL fraction (AUC) on the two separate occasions ranged from 3 to 6% and that for retinyl palmitate formed from 4.0 to 9.0% (Table 4). The data suggest that the response to a 40 mg \( \beta \)-carotene dose is surprisingly constant within an individual and reproducible over time.

Discussion

In intestinal cells, \( \beta \)-carotene can either be secreted directly into chylomicrons, or can be converted to retinol, which is esterified with palmitic acid for export in chylomicrons (Goodman et al. 1966). In the present study, we measured the response in the TRL fraction for \( \beta \)-carotene and its conversion to retinyl palmitate in both males and females.
Fig. 3. Concentrations of retinyl palmitate in postprandial triacylglycerol-rich lipoproteins (TRL) from three individual subjects who received 40 mg encapsulated β-carotene with a standard meal on two separate occasions approximately 4 months apart. (a), Subject no.1, male; (b), subject no. 2, male; (c), subject no. 7, female. Blood was collected at hourly intervals for 8 h on the first occasion and 12 h on the second occasion while subjects fasted. Curves shown are smoothed plots through the specific points at the times shown.

after a 40 mg oral dose of β-carotene. There was considerable variability in response curves between individuals with no difference observed between the sexes.

The maximal concentration in the β-carotene response curve (Fig. 1) was observed at the fourth–fifth hour postprandially. The time of the maximal response was similar to that documented by Traber et al. (1994) following supplementation with 60 mg of β-carotene, who reported it between the third and sixth hours. Van Vliet & Van den Berg (1995) gave men a 15 mg β-carotene supplement and observed a peak in the TRL response at approximately the fifth hour with β-carotene concentrations in the TRL fraction falling to approximately 10 nmol/l by 8 h. In our work, β-carotene was present in the TRL fraction for approximately the same amount of time, for at the eighth hour postprandially β-carotene concentrations in the TRL fraction were only just above baseline in both sexes at approximately 15 nmol/l. It appears that the higher dose of β-carotene (40 mg) given did not affect the disappearance of the carotenoids from the TRL fraction. However, experiments in three more subjects (two males and one female) were done to investigate the amount of β-carotene remaining in the TRL fraction between 8 and 12 h postprandially. In all three subjects, the β-carotene
concentration in the TRL fraction after the tenth hour had fallen to < 10 nmol/l (Fig. 2).

In an attempt to decrease the large inter-individual variation in the β-carotene AUC data, the same data were adjusted using the TRL responses for triacylglycerols. However this adjustment did not decrease inter-individual variation; if anything variation in retinol responses increased (Table 2). As reported by Erdman et al. (1993) the time-courses of appearance and the half-lives of triacylglycerols and carotenoids in chylomicrons have been shown to be nearly identical, therefore the correction of carotenoid levels in chylomicrons for triacylglycerol levels is unnecessary (Gartner et al. 1996).

The absorptive capacities for the different carotenoids and their products were estimated using the AUC method (Table 2). No difference was observed between men and women for either the total β-carotene present in the TRL fraction or the amount of retinyl palmitate formed. However, for retinyl palmitate, the median AUC was approximately 25% higher in males 101 (interquartile range 70–120) nmol/h/l than in females 75 (interquartile range 38–87) nmol/h/l. This apparent slight difference in conversion of β-carotene to retinyl palmitate between the sexes may be due to a more efficient chylomicron-remnant clearance in women. There is evidence for sex-related differences in post-absorptive chylomicron-remnant metabolism which may be related to the effects of female hormones on lipid and lipoprotein metabolism (Johnson et al. 1992). Johnson et al. (1992) also found this sex difference in plasma retinyl esters with peak rise and AUC being lower in females than males. Alternatively, males may have a more efficient enzyme activity for conversion of β-carotene to retinyl palmitate to match the higher vitamin A requirements for males.

In our studies, in order to estimate the absorption of intestinal β-carotene and conversion to retinyl palmitate we assumed exogenous cleavage of β-carotene to retinol, giving a mean total amount of β-carotene absorbed in both sexes from the 40 mg capsule of 1.34 mg. That is, 0.83 mg was present as β-carotene and 0.51 mg appeared as retinol. The comparable values obtained by Van Vliet & Van den Berg (1995) from a 15 mg dose, were 0.65 mg β-carotene and 1.1 mg retinol or a total of 1.7 mg total β-carotene in the TRL fraction. While the amount of β-carotene which appeared in the chylomicrons was relatively similar in the two studies, the conversion of β-carotene to retinyl palmitate was two fold higher in the study of Van Vliet & Van den Berg (1995) than in our own. The finding that 64% of the total β-carotene absorbed was converted to retinol palmitate is similar to those reported by Goodman et al. (1966) and Bloomstrand & Werner (1967) who showed that after feeding labelled β-carotene, 60–75% of absorbed β-carotene was converted to retinol. These data suggest that the human intestine possesses only a limited absorptive capacity for absorption of unchanged dietary β-carotene. In our study, only 42% of the total β-carotene was converted to retinyl palmitate. All the volunteers (Table 2) displayed absolute AUC concentration for total β-carotene values within the range shown by Van Vliet & van den Berg (1995) (31–328 nmol/h/l), with the exception of one subject who was just outside (subject 1, AUC 382 nmol/h/l).

However, the mean AUC for retinyl palmitate in our studies was two times smaller that than of Van Vliet & Van den Berg (1995). A possible reason for this difference in retinyl palmitate conversion may be that our volunteers had a lower capacity or need to convert β-carotene to retinyl palmitate. Alternatively, retinol may have been transferred to other lipoproteins or taken up quickly by the liver (i.e. may have a different t1/2 in TRL). From these studies, it appears that < 2 mg is absorbed from a β-carotene supplement irrespective of supplement dose suggesting that intestinal absorption of β-carotene may be a saturable process. A recent study by Novotny et al. (1995) using a multi-compartment model predicted 22% absorption from a 40 mg oral β-carotene dose, 17.8% as β-carotene and 4.2% as retinoid. However, this model was constructed using data from a single subject and would appear to be an overestimation of β-carotene absorption; alternatively the assumptions for β-carotene turnover in our study were too low.

The AUC response curves in the TRL fraction for the six subjects who consumed β-carotene, lutein and lycopene are shown in Table 3. Each dose of β-carotene (40 mg), lutein (31.2 mg), and lycopene (38 mg) was administered on a separate occasion, 14 d apart, each individual undergoing the same protocol as outlined before. Separate feeding studies were done to eliminate possible competition between the carotenoids for absorption (Micoczi et al. 1992; Kostic et al. 1995). Total β-carotene concentration in the TRL fraction was significantly higher than lutein (P < 0.05) and although the AUC for lycopene was lower than total β-carotene this was not significant. After correction for their slightly smaller doses and presented as a percentage of total β-carotene, a significant difference (P < 0.05) between β-carotene and lutein concentration still remained. Differences seen between the polar and non-polar carotenoids may be due to the physical positioning of the different carotenoids within the lipoproteins which would cause them to be transported and cleared differently. In our studies, lutein peak concentration was at an earlier stage postprandially (2 h) than the other two carotenoids. β-Carotene and lycopene peaked between the fourth and sixth hours postprandially. It may explain the lower AUC found for lutein, if lutein was taken up and cleared from the TRL fraction into other serum lipoproteins more quickly than the other more hydrophobic carotenoids. A recent report by Bierer et al. (1995) in prernuminate calves reported that the xanthophylls (lutein and canthaxanthin) peaked earlier and were also cleared more quickly from serum than the hydrocarbon carotenoids (β-carotene, α-carotene, lycopene). Lutein or lycopene in these calf studies could not be detected in the TRL fraction, however the polar and non-polar carotenoids appeared in equal amounts in serum. Also, Kostic et al. (1995) observed that the mean serum AUC for lutein was approximately twice that for an equimolar dose of β-carotene in oil. In our studies we only viewed the concentration of the carotenoids in the TRL fraction, the transfer of the carotenoids to other lipoprotein fractions or into the tissues is not known so it is not possible to compare directly the results obtained from serum with those of the TRL fractions.
However inter-individual variation was large. To investigate the reproducibility of the \( \beta \)-carotene response curves and the conversion to retinyl palmitate in the TRL fraction, response curves for \( \beta \)-carotene and retinyl palmitate were studied in subjects given a \( 40 \) mg \( \beta \)-carotene dose on two separate occasions, to investigate the reproducibility of the \( \beta \)-carotene response curves and the conversion to retinyl palmitate in the TRL fraction (Figs. 2 and 3). Intra-subject variation was small, ranging between 4 and 9\% for retinyl palmitate formation, and a 3–6\% variation was observed for total \( \beta \)-carotene absorbed (Table 4). In contrast, Van Vliet & Van den Berg (1995) carried out a repeat experiment 16 d after their first study. Intra-subject differences were not documented, however their results before adjustment for triacylglycerols showed intergroup differences of 23\% for retinyl palmitate formed and 33\% for \( \beta \)-carotene absorbed.

In conclusion, this study demonstrated that only small amounts of \( \beta \)-carotene appeared in the TRL fraction following a 40 mg dose, suggesting that intestinal absorption of \( \beta \)-carotene is a saturable process. The polarity of a carotenoid may determine the uptake and clearance of carotenoids from the intestine and response curves in the TRL fraction. Response curves for \( \beta \)-carotene and retinyl palmitate were highly reproducible within individuals, however inter-individual variation was large. To investigate the large inter-individual variations future work is required to investigate other factors affecting carotenoid absorption, transport and metabolism.

### Acknowledgements

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