Intestinal absorption of β-carotene ingested with a meal rich in sunflower oil or beef tallow: postprandial appearance in triacylglycerol-rich lipoproteins in women1–4

Xixuan Hu, Ronald J Jandacek, and Wendy S White

ABSTRACT

Background: Evidence indicates that different types of fat have different effects on the postprandial plasma triacylglycerol response. Therefore, the type of fat may influence the appearance of β-carotene in postprandial triacylglycerol-rich lipoproteins, which is used as an indicator of intestinal β-carotene absorption.

Objective: We compared in female subjects the appearance of β-carotene in plasma triacylglycerol-rich lipoproteins after β-carotene was ingested with a meal containing sunflower oil or beef tallow.

Design: Women (n = 11) each ingested 2 different vitamin A–free, fat-rich meals that were supplemented with β-carotene (47 μmol) and contained equivalent amounts (60 g) of sunflower oil or beef tallow. Blood samples were collected hourly from 0 to 10 h; additional samples were collected at selected intervals until 528 h. In a subgroup of the women (n = 7), plasma chylomicrons and 3 subfractions of VLDLs were separated by cumulative rate ultracentrifugation.

Results: The appearance of β-carotene in chylomicrons and in each VLDL subfraction was lower after ingestion of the meal containing sunflower oil than after ingestion with the meal containing beef tallow (P < 0.03). In chylomicrons, the area under the concentration-versus-time curve (AUC) for β-carotene was 38.1 ± 13.6% lower (P < 0.03); in contrast, the AUC for triacylglycerol was higher (P < 0.05) after the sunflower-oil-rich meal than after the beef-tallow-rich meal.

Conclusions: Ingestion of β-carotene with a meal rich in sunflower oil as compared with a meal rich in beef tallow results in lower appearance of β-carotene and greater appearance of triacylglycerol in triacylglycerol-rich lipoproteins.

INTRODUCTION

Dietary fat is a major determinant of the intestinal absorption of β-carotene (1), partly because the intestine is unable to secrete significant amounts of triacylglycerol-rich lipoproteins in the absence of dietary fat (2). Chylomicrons are the most triacylglycerol-rich of the plasma triacylglycerol-rich lipoproteins (3); they transport ingested β-carotene and its major cleavage product, retinyl esters, from the small intestine via the lymph and blood to the liver and tissues (4). Although it is known that dietary fat is needed for intestinal absorption of β-carotene, there has been little systematic study of the effects of the amount or nature of dietary fat on the appearance of β-carotene in plasma triacylglycerol-rich lipoproteins, which is used as an indicator of intestinal absorption (5).

Few human studies have addressed the effects of the nature of dietary fat on the amount and composition of postprandial as opposed to fasting lipoproteins, despite the fact that most individuals spend the majority of each 24-h period in the postprandial state (6). On the basis of a small number of human studies, the general conclusion has been that meals consisting predominantly of saturated, monounsaturated, or n–6 polyunsaturated fatty acids elicit a similar postprandial lipemic response (6, 7), whereas meals contributing significant amounts of long-chain n–3 fatty acids (eg, fish oil) produce an attenuated lipemic response (8). However, early investigators reported greater postprandial lipemia after ingestion of vegetable oils rich in linoleic or linolenic acids than after consumption of more saturated fats such as trimyristin (9) and butter (10). In a crossover trial published in 1995, the plasma triacylglycerol response was enhanced > 3-fold after an oral fat load containing corn oil relative to a fat load containing beef tallow (11). These

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3Supported by The Procter & Gamble Company and by Hatch Act and State of Iowa funds. The β-carotene beadlets and β-cryptoxanthin were generously donated by Vishwa N Singh and Roche Vitamins Inc; the lutein by Kemin Industries; the beef tallow by AC Humko; the non-vitamin-fortified, liquid nonfat milk by Anderson Erickson Dairy Co; and the non-vitamin-fortified, spray-dried nonfat milk by Associated Milk Producers Inc.
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findings highlight the need for additional human studies regarding the effects of the qualities of the fat components of meals on the gastrointestinal processing of dietary fat.

The effects of meals with different fatty acid compositions on the postprandial lipemic response suggest potential effects on the intestinal absorption of fat-soluble dietary components such as β-carotene. In 1978, Hollander and Ruble (12) reported distinct effects of long-chain fatty acids of different saturation on the absorption of β-carotene from an intestinal perfusate in rats. If analogous effects could be shown in humans by using ingested fats with different fatty acid compositions, new insight would be provided regarding mechanisms of intestinal β-carotene absorption. Such insight could be applied to optimize the provitamin A and other effects of dietary β-carotene through modification of dietary fat. The objective of the present study was to compare, in healthy young women, the appearance of β-carotene in plasma triacylglycerol-rich lipoproteins after ingestion with a polyunsaturated fat, sunflower oil, and a more saturated fat, beef tallow.

SUBJECTS AND METHODS

Subjects

Fourteen healthy women aged 19–30 y were enrolled in the study. Of these women, 2 were found to have veins not amenable to phlebotomy and a third was not available to complete the second period of the study. The characteristics of the remaining 11 subjects who completed the 2 periods of the study are presented in Table 1.

Subjects underwent a screening procedure that included a health and lifestyle questionnaire, physical examination, complete blood count, and blood chemistry profile. Criteria for exclusion were current or recent (previous 12 mo) cigarette smoking, current or planned pregnancy, current or recent (previous 12 mo) use of oral contraceptive agents or contraceptive implants, current or recent (previous 1 mo) use of medications that may affect lipid absorption or transport (including antibiotics), current use of vitamin or mineral supplements, frequent consumption of alcoholic beverages (> 1 drink/d), hyper- or hypothyroidism diagnosed by measuring serum thyroxine and thyroid-stimulating hormone concentrations, hyperlipidemia diagnosed by determining the plasma lipid and lipoprotein profile, and vegetarianism. Also excluded were those who had a history of anemia or excessive bleeding, chronic disease, eating disorders, lactose intolerance, lipid malabsorption or intestinal disorders, photosensitivity disorders, and menstrual cycle irregularities or abnormalities. Percent-age body fat at the beginning of the study was determined by dual-energy X-ray absorptiometry (QDR 2000; Hologic Inc, Waltham, MA). Informed consent was obtained from all subjects and the study procedures were approved by the Human Subjects Research Review Committee of Iowa State University.

Experimental diet

Subjects were instructed to avoid consumption of carotenoid-rich fruits and vegetables and vitamin A–rich foods for 4 d before each study period; they were given a list of these foods to be avoided. During the study periods, subjects consumed a controlled, low-carotenoid, low–vitamin A diet for 2 d before and 4 d after dosing. The diet consisted of conventional foods except for non-vitamin-fortified, nonfat milk (Anderson Erickson Dairy Co, Des Moines, IA). A single daily menu of weighed food portions was provided. The meals were prepared and consumed in the Human Nutrition Metabolic Unit of the Center for Designing Foods to Improve Nutrition at Iowa State University, except for the carry-out lunches and evening snacks on weekdays. Adherence to the experimental diet was monitored by written self-report and by analysis with HPLC of fasting plasma carotenoid concentrations. Duplicate aliquots of 24-h diet composites from each of the 2 study periods were analyzed for carotenoids, vitamin A, and α-tocopherol contents. Extraction of these analytes was performed by following a protocol that was described previously (13). On average across 2 study periods, the daily diet provided 299.8 ± 24.6 μg lutein, 79.7 ± 6.1 μg β-cryptoxanthin, 30.0 ± 10.8 μg β-carotene, no detectable α-carotene or lycopene, 53.4 ± 8.4 μg vitamin A, and 8.9 ± 0.8 mg α-tocopherol. The macronutrient composition of the diet was estimated by using NUTRITIONIST V software (N-Squared Computing Inc, Salem, OR). The dietary energy (9.4 MJ/d) was distributed as 14% of total energy from protein, 58% from carbohydrate, and 28% from fat.

Test meals

Subjects ingested β-carotene (47 μmol) with each of 2 vitamin A–free test meals, which were in the form of a liquid emulsion containing sunflower oil or beef tallow. The test meals contained 35 g sucrose (California & Hawaiian Sugar Co Inc, Crockett, CA); 25 g non-vitamin-fortified, spray-dried, nonfat dry milk (Associated Milk Producers Inc, Mason City, IA); 60 mL water; and 60 g fat (70% of total energy) in the form of either sunflower oil (Hunt-Wesson Inc, Fullerton, CA) or refined beef tallow (AC Humko, Denver). The test meals were prepared as described by Sakr et al (14). The composition of the test meals was analyzed by Covance Laboratories Inc, Madison, WI (Table 2). Total protein was analyzed by the Dumas method as modified by King-Brink and Sebranek (15); total fat was analyzed by the Association of Official Analytical Chemists (AOAC) acid hydrolysis method (16) and gravimetric method (17); cholesterol was analyzed by the AOAC direct saponification–gas chromatographic method (18); moisture was analyzed by an AOAC method (19); and total carbohydrate was measured by a US Department of Agriculture method (20). Fatty acid distribution was analyzed by gas-liquid chromatography (21, 22; Table 3). Carotenoids, retinoids, and tocopherols were extracted from aliquots of the test

### Table 1

Subject characteristics at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>23.6 ± 4.0 (19–30)</td>
</tr>
<tr>
<td>BMI (in kg/m²)</td>
<td>21.6 ± 2.4 (20.0–28.3)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>31.0 ± 2.4 (22.0–48.5)</td>
</tr>
<tr>
<td>Fasting plasma concentrations</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.43 ± 0.16 (3.46–5.11)</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>0.78 ± 0.08 (0.33–1.35)</td>
</tr>
<tr>
<td>α-Carotene (μmol/L)</td>
<td>0.096 ± 0.054 (0.016–0.209)</td>
</tr>
<tr>
<td>β-Carotene (μmol/L)</td>
<td>0.345 ± 0.123 (0.128–0.558)</td>
</tr>
<tr>
<td>β-Cryptoxanthin (μmol/L)</td>
<td>0.199 ± 0.070 (0.084–0.312)</td>
</tr>
<tr>
<td>Lutein (μmol/L)</td>
<td>0.176 ± 0.079 (0.091–0.339)</td>
</tr>
<tr>
<td>Lycopene (μmol/L)</td>
<td>0.837 ± 0.303 (0.271–1.251)</td>
</tr>
<tr>
<td>Retinol (μmol/L)</td>
<td>1.45 ± 0.28 (1.00–1.92)</td>
</tr>
<tr>
<td>α-Tocopherol (μmol/L)</td>
<td>23.0 ± 4.1 (18.0–30.6)</td>
</tr>
</tbody>
</table>

*SEM with range in parentheses; n = 11*
TABLE 2
Composition of test meals

<table>
<thead>
<tr>
<th>Composition</th>
<th>Test meals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef-tallow–rich</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>9.4 ± 0.3</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>60.5 ± 1.7</td>
</tr>
<tr>
<td>Total carbohydrate (g)</td>
<td>52.7 ± 2.2</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>81.4 ± 0.6</td>
</tr>
<tr>
<td>Moisture (g)</td>
<td>55.8 ± 0.2</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>3314 ± 30</td>
</tr>
</tbody>
</table>

†± SEM of duplicate samples.

meals and analyzed as described previously (13). The sunflower-oil–rich meal contained 27.6 mg α-tocopherol and the beef-tallow–rich meal contained no detectable α-tocopherol. There were no detectable carotenoids or vitamin A in either test meal.

The β-carotene was added as water-dispersible beadlets containing 10% (by wt) synthetic β-carotene (Roche Vitamins Inc, Parsippany, NJ). For preparation of the test meals, which provided 25 mg (47 μmol) β-carotene, 250 mg of the beadlets were dissolved in 60 mL warm (40°C) nonfat milk prepared from water, sucrose, and nonfat dry milk. The oil or fat was then added and the mixture was agitated to produce an emulsion.

Study protocol

Each subject ingested β-carotene (47 μmol) with each of the 2 test meals. The test meals were ingested in random order and were separated by a washout period of ≥4 wk, during which subjects consumed their habitual diets. The duration of the washout period was based on a previous investigation in which we found that plasma β-carotene concentrations returned to baseline after 6 h blood draw. Additional blood samples were drawn by venipuncture from the antecubital vein after an overnight fast at 0, 2, 4, 6, 8, and 10 h after ingestion of the test meal were used immediately for lipoprotein fractionation. Cumulative-rate ultracentrifugation was used to isolate chylomicrons, 3 VLDL sub-

TABLE 3
Fatty acid composition of the test meals

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Test meals</th>
<th>% by wt of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef-tallow–rich</td>
<td>Sunflower-oil–rich</td>
</tr>
<tr>
<td>8:0</td>
<td>0.7 ± 0.1</td>
<td>&lt;0.1 ± 0.0</td>
</tr>
<tr>
<td>14:0</td>
<td>3.4 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>14:1</td>
<td>0.8 ± 0.0</td>
<td>&lt;0.1 ± 0.0</td>
</tr>
<tr>
<td>15:0</td>
<td>0.7 ± 0.1</td>
<td>&lt;0.1 ± 0.0</td>
</tr>
<tr>
<td>16:0</td>
<td>24.0 ± 0.2</td>
<td>6.5 ± 0.1</td>
</tr>
<tr>
<td>16:1</td>
<td>3.3 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>17:0</td>
<td>1.7 ± 0.0</td>
<td>&lt;0.1 ± 0.0</td>
</tr>
<tr>
<td>18:0</td>
<td>16.3 ± 0.3</td>
<td>4.2 ± 0.0</td>
</tr>
<tr>
<td>18:1</td>
<td>44.9 ± 0.4</td>
<td>18.7 ± 0.0</td>
</tr>
<tr>
<td>18:2</td>
<td>2.0 ± 0.1</td>
<td>68.6 ± 0.1</td>
</tr>
<tr>
<td>18:3</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>18:4</td>
<td>0.6 ± 0.1</td>
<td>&lt;0.1 ± 0.0</td>
</tr>
<tr>
<td>20:0</td>
<td>0.3 ± 0.0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>20:1</td>
<td>0.5 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>22:0</td>
<td>&lt;0.1 ± 0.0</td>
<td>0.7 ± 0.0</td>
</tr>
<tr>
<td>24:0</td>
<td>&lt;0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>SFA</td>
<td>46.9 ± 0.4</td>
<td>12.0 ± 0.1</td>
</tr>
<tr>
<td>MUFA</td>
<td>50.5 ± 0.4</td>
<td>19.1 ± 0.1</td>
</tr>
<tr>
<td>PUFA</td>
<td>2.7 ± 0.1</td>
<td>68.9 ± 0.1</td>
</tr>
</tbody>
</table>

†± SEM of duplicate samples. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
equal volume of absolute ethanol containing 0.1g butylated hydroxytoluene (BHT)/L and retinyl acetate as the internal standard. Samples were then extracted twice with hexane containing 0.1 g BHT/L and the combined hexane layers were evaporated to dryness under vacuum. The residues were reconstituted with ethyl ether and mobile phase A (1:3 by vol) and 20-μL aliquots were injected into the HPLC system. The components of the HPLC system consisted of the 717Plus autosampler with temperature control set at 5°C, two 510 solvent-delivery systems, and the 996 photodiode array detector (Waters Corporation, Milford, MA). The system operated with MILLENIUM 2010 Chromatography Manager software (version 2.10; Waters Corporation). Data were collected at 290, 325, and 453 nm. Separation of analytes was performed on a 5-μm C30 Carotenoid Column (4.6 × 250 mm; YMC Inc, Wilmington, NC) protected by a precolumn packed with the same stationary phase. Analytes were eluted by using a linear mobile-phase gradient from 100% methanol (1 g ammonium acetate/L) to 100% methyl-tert-butyl ether (MTBE) over 30 min, as modified from the method of Sander et al (27). The flow rate was 1.0 mL/min. Solvents were HPLC grade; the methanol, MTBE, and ammonium acetate were purchased from Fisher Scientific (Chicago). The mobile phase was filtered (Nylon-66 filter, 0.2 μm; Rainin Instruments Co, Woburn, MA) and degassed before use.

Calibration curves were generated from the ratio of the peak height of the analyte standard to the peak height of the retinyl acetate internal standard plotted against the analyte concentration. Retinol, retinyl acetate, α-tocopherol, α-carotene, and β-carotene standards were purchased from Fluka Chemical (Ronkonkoma, NY) and lycopene and retinyl palmitate standards were purchased from Sigma Chemical (St Louis). Lutein and β-cryptoxanthin were supplied by Kemin Industries (Des Moines, IA) and Roche Vitamins, respectively. Accuracy and precision of the analyses were verified by using a standard reference material (SRM 968b, Fat-Soluble Vitamins in Human Serum) from the National Institute of Standards and Technology (Gaithersburg, MD). Quality control included routine analysis of a plasma pool; interassay CVs were <5% for carotenoids, retinol, and α-tocopherol. For the lipoprotein data, the presented concentrations (μmol/L) are based on the original volume of plasma used for isolation of the lipoproteins.

The triacylglycerol contents of total plasma and plasma triacylglycerol-rich lipoproteins were determined enzymatically by using a commercial assay (GPO-Trinder; Sigma Diagnostics). The cholesterol contents of total plasma, plasma triacylglycerol-rich lipoproteins, LDLs, and plasma infranate were also determined enzymatically by using a commercial assay (Total Cholesterol; Sigma Diagnostics). The accuracy of the analyses was determined by using Accutrol Unassayed Chemistry Controls, Normal (Sigma Diagnostics).

Data analysis

The data were analyzed by repeated-measures analysis of variance (ANOVA) as a 2-period crossover design. Beef tallow and sunflower oil were included as treatments and individual time points were included as a split-plot factor (28). The changes from baseline for plasma β-carotene concentration and the contents of β-carotene, retinyl palmitate, triacylglycerol, and cholesterol in triacylglycerol-rich lipoproteins were quantified as the area under the concentration-versus-time curve (AUC) calculated by trapezoidal approximation (29).

Fractional absorption of the β-carotene in the test meal was estimated according to van Vliet et al (5):

\[
\text{Fractional absorption} = \left( \frac{\ln 2}{t_{1/2}} \right) \times \frac{\text{[calculated oral AUC}}\times \text{mass} \times \text{plasma volume}}{\text{oral dose}}
\]

where it was assumed that the t_{1/2} of β-carotene and retinyl palmitate was equivalent to that of chylomicrons (0.192 h or 11.5 min), and plasma volume (mL) = 927 + (31.47 × body wt in kg).

**RESULTS**

Postprandial appearance of β-carotene and retinyl palmitate

The postprandial appearance of β-carotene in plasma triacylglycerol-rich lipoproteins is shown in Figure 1. The appearance of β-carotene in each subfraction (chylomicrons, VLDL_{A}, VLDL_{B}, and VLDL_{C}) was lower after ingestion with a meal rich in sunflower oil than after ingestion with a meal rich in beef tallow, according to repeated-measures ANOVA (P < 0.03). The postprandial appearance of retinyl palmitate, the major cleavage product of β-carotene, in plasma triacylglycerol-rich lipoproteins is shown in Figure 2. The appearance of retinyl palmitate in chylomicrons was not significantly different (P = 0.26) after the 2 test meals, whereas that in VLDL_{A} and VLDL_{B} was lower after ingestion of β-carotene with a meal rich in sunflower oil (P < 0.04). In chylomicrons, when the peak appearance of retinyl palmitate was compared within subjects, the peak concentration was 27.2 ± 10.0% lower after ingestion of the sunflower-oil–rich meal than after ingestion of the beef-tallow–rich meal; however, this difference was not significant (P = 0.17). Overall, the data indicate a lower appearance of both β-carotene and the retinyl palmitate cleavage product in triacylglycerol-rich lipoproteins when β-carotene was ingested with a sunflower-oil–rich meal.

The AUC values for β-carotene and retinyl palmitate in the chylomicron fraction of each subject are shown in Table 4. In chylomicrons, the AUC for β-carotene was 38.1 ± 13.6% lower (P < 0.03) but the AUC for retinyl palmitate was not significantly different (P = 0.29) after the sunflower-oil–rich meal than after the beef-tallow–rich meal. The sum of the AUC values for β-carotene and retinyl palmitate, which is used as an indicator of total β-carotene absorption (30), was lower after the sunflower-oil–rich meal than after the beef-tallow–rich meal (P < 0.04). Of the absorbed β-carotene, 44.3% of that ingested with sunflower oil and 35.7% of that ingested with beef tallow was converted to retinyl palmitate (P = 0.07).

The appearance of β-carotene in lipoproteins with density > 1.006 kg/L was lower after the meal rich in sunflower oil than after the meal rich in beef tallow by repeated-measures ANOVA (P < 0.01) (Figure 3). When compared within subjects, the peak β-carotene content during the 10-h period was lower after ingestion with sunflower oil than after ingestion with beef tallow by...
44.1 ± 11.4% in lipoproteins with density 1.010–1.020 kg/L, by 62.3 ± 3.1% in LDLs, by 55.7 ± 9.8% in lipoproteins with density 1.060–1.090 kg/L, and by 53.8 ± 3.4% in infranate (P < 0.01). At 10 h, the distribution of β-carotene resulted in its content being highest in plasma infranate, followed by LDL.

Thus, there was a lower appearance of β-carotene in lipoproteins with densities ≤ 1.006 kg/L and > 1.006 kg/L after ingestion with a sunflower-oil–rich meal.

The plasma concentration-versus-time curve for β-carotene is characterized by an early peak at 5–8 h followed by a sus-

**FIGURE 1.** Mean (±SEM) change in β-carotene content from baseline (fasting) in plasma triacylglycerol-rich lipoproteins after subjects ingested 47 μmol β-carotene with a meal containing either beef tallow (○) or sunflower oil (●); n = 7. The P values shown correspond to the main effect of the test meals as analyzed by repeated-measures ANOVA.

**FIGURE 2.** Mean (±SEM) appearance of retinyl palmitate, the major cleavage product of β-carotene, in plasma triacylglycerol-rich lipoproteins after subjects ingested 47 μmol β-carotene with a meal containing either beef tallow (○) or sunflower oil (●); n = 7. The P values shown correspond to the main effect of the test meals as analyzed by repeated-measures ANOVA.
tained peak at 24–48 h (Figure 4). These 2 peaks reflect the dynamics of the incorporation of $\beta$-carotene into plasma lipoproteins (23). The overall plasma appearance of $\beta$-carotene was lower after the sunflower-oil–rich meal than after the beef-tallow–rich meal by repeated-measures ANOVA ($P < 0.05$). The within-subject comparison of the plasma $\beta$-carotene AUC values for 0–96 h after ingestion of the 2 test meals is shown in Table 5. The 0–96-h period represents the duration of the controlled, low-carotenoid diet after ingestion of the test meal. The AUC values varied greatly, ranging from 34.9 to 102.3 $\mu$mol · h/L after ingestion of the sunflower-oil–rich meal and from 7.2 to 169.0 $\mu$mol · h/L after ingestion of the beef-tallow–rich meal. In subjects 1–9, the plasma $\beta$-carotene AUC for 0–96 h was $41.5 \pm 7.6\%$ lower (average of the within-subject differences) after the meal containing sunflower oil as compared with the meal containing beef tallow. In the remaining 2 subjects, the plasma $\beta$-carotene AUC was higher after the meal containing sunflower oil. Unlike subjects 1–9, subjects 10 and 11 were not normolipidemic at baseline; subject 10 had a low plasma triacylglycerol concentration (0.328 mmol/L) and subject 11 had a high plasma LDL-cholesterol concentration (3.39 mmol/L). Overall, for all of the 11 subjects, the plasma $\beta$-carotene AUC was an average of $30.3 \pm 14.7\%$ lower after ingestion of the sunflower-oil–rich meal ($P < 0.07$).

**Fractional absorption of $\beta$-carotene**

We assumed that 1 mol $\beta$-carotene is converted to 1 mol retinyl palmitate during intestinal absorption (eccentric cleav-

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**TABLE 4**

<table>
<thead>
<tr>
<th>Subject</th>
<th>$\beta$-carotene AUC $\mu$mol · h/L</th>
<th>Retinyl palmitate AUC $\mu$mol · h/L</th>
<th>Total $\beta$-carotene AUC $\mu$mol · h/L</th>
<th>Retinyl palmitate AUC as % of $\beta$-carotene AUC</th>
<th>$F$-value</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef tallow</td>
<td>Sunflower oil</td>
<td>Beef tallow</td>
<td>Sunflower oil</td>
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<td>Sunflower oil</td>
</tr>
<tr>
<td>1</td>
<td>0.224</td>
<td>0.270</td>
<td>0.118</td>
<td>0.098</td>
<td>0.342</td>
<td>0.368</td>
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<tr>
<td>2</td>
<td>0.499</td>
<td>0.136</td>
<td>0.322</td>
<td>0.239</td>
<td>0.821</td>
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<td>3</td>
<td>0.599</td>
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<td>0.136</td>
<td>0.109</td>
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<td>0.808</td>
<td>0.303</td>
<td>0.172</td>
<td>0.153</td>
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<td>0.114</td>
<td>0.663</td>
<td>0.273</td>
<td>0.980</td>
<td>0.387</td>
</tr>
<tr>
<td>7</td>
<td>0.388</td>
<td>0.400</td>
<td>0.356</td>
<td>0.398</td>
<td>0.744</td>
<td>0.798</td>
</tr>
<tr>
<td>Mean $\pm$ SEM</td>
<td>$0.490 \pm 0.075$</td>
<td>$0.268 \pm 0.041^2$</td>
<td>$0.280 \pm 0.072$</td>
<td>$0.214 \pm 0.040$</td>
<td>$0.770 \pm 0.081$</td>
<td>$0.482 \pm 0.060^2$</td>
</tr>
</tbody>
</table>

$^1$ Sum of the $\beta$-carotene AUC and retinyl palmitate AUC.

$^2$ Significantly different from beef tallow, $P < 0.05$ (paired Student’s $t$ test).

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**Figure 3.** Mean ($\pm$SEM) change in $\beta$-carotene content from baseline (fasting) in plasma lipoproteins with density ($d$) $> 1.006$ kg/L after subjects ingested 47 $\mu$mol $\beta$-carotene with a meal containing either beef tallow (○) or sunflower oil (●); $n = 7$. The $P$ values shown correspond to the main effect of the test meals as analyzed by repeated-measures ANOVA.
age) (31) and we calculated the fraction of the β-carotene dose absorbed by each subject according to van Vliet et al (5). When compared within subjects, the mean fractional absorption was 6.4 ± 2.2% higher after ingestion of the beef-tallow–rich meal than after ingestion of the sunflower-oil–rich meal (P < 0.03 by paired Student’s t test). The fractional absorption ranged from 8.0% to 16.7% (2.0–4.2 mg) after ingestion of the sunflower-oil–rich meal and from 10.0% to 22.3% (2.5–5.6 mg) after ingestion of the beef-tallow–rich meal. The estimated average absorption efficiencies (10.9% for sunflower oil and 17.3% for beef tallow) were comparable with the range of values reported by other authors (2.5–17.0%) (5, 30).

Lipemic response

The changes from fasting in the triacylglycerol content of triacylglycerol-rich lipoproteins at 2, 4, 6, 8, and 10 h after ingestion of the test meals are shown in Figure 5. The triacylglycerol response in chylomicrons and in VLDLc was higher after the sunflower-oil–rich meal than after the beef-tallow–rich meal by repeated-measures ANOVA (P < 0.05). The VLDLc fraction is thought to contain the majority of hepatic VLDLs (32). In contrast, the change in the triacylglycerol concentration in total plasma (Figure 6) was similar after the 2 test meals (P = 0.80). The changes in cholesterol content in triacylglycerol-rich lipoproteins, LDLs, plasma infranate, and total plasma (data not shown) were not significantly different after ingestion of the 2 test meals.

DISCUSSION

We used the postprandial appearance of β-carotene and its major cleavage product, retinyl palmitate, in plasma triacylglycerol-rich lipoproteins as measures of apparent intestinal β-carotene absorption and cleavage. Most previous investigations of intestinal β-carotene absorption in humans have relied on the change in β-carotene concentration in total plasma as the outcome measure (1, 33, 34). However, plasma chylomicrons offer an important advantage as a vehicle for assessing intestinal β-carotene absorption; this advantage is the ability to distinguish newly absorbed β-carotene from that which has cycled into and out of the liver (5). The utility of the total plasma triacylglycerol-rich lipoprotein fraction as a vehicle for assessing intestinal absorption and cleavage of β-carotene has been studied well in humans (5, 30). The underlying assumption is that the triacylglycerol-rich lipoprotein fraction contains primarily intestinally derived lipoproteins (chy-
lomicrons and their remnants) and some liver-derived lipoproteins (VLDLs). However, in the current study and previously (23), we have shown postprandial accumulations of \(\beta\)-carotene in large VLDLs (VLDL\(A\)) that coincide with and are of similar magnitude to those in chylomicrons. In triacylglycerol-rich lipoproteins, the postprandial increase in apolipoprotein B-100 was shown to exceed that of apolipoprotein B-48, the integral apolipoprotein in chylomicrons (35), and to be confined to large VLDLs (36, 37). The postprandial increase in apolipoprotein B-100 in triacylglycerol-rich lipoproteins has been attributed to accumulation of hepatogenous VLDLs as a result of saturation of lipoprotein lipase by chylomicrons (35, 36). Therefore, the use of the total plasma triacylglycerol-rich lipoprotein fraction may overestimate intestinal \(\beta\)-carotene absorption. We have subfractionated triacylglycerol-rich lipoproteins into particles of varying size and composition in an effort to better distinguish particles of exogenous and endogenous origin.

We showed that the apparent intestinal absorption of \(\beta\)-carotene, as measured by appearance in chylomicrons, was lower when \(\beta\)-carotene was ingested with a sunflower-oil–rich meal than when it was ingested with a beef-tallow–rich meal. Similarly, the postprandial appearance of \(\beta\)-carotene in the other subfractions of triacylglycerol-rich lipoproteins (VLDL\(A\), VLDL\(B\), and VLDL\(C\)) (Figure 1) and in lipoproteins with density > 1.006 kg/L (Figure 3) was lower after the sunflower-oil–rich meal. The consistency of the treatment difference across chylomicrons and other plasma lipoproteins indicates an effect on intestinal \(\beta\)-carotene absorption. We have subfractionated triacylglycerol-rich lipoproteins into particles of varying size and composition in an effort to better distinguish particles of exogenous and endogenous origin.

The triacylglycerol response in chylomicrons and in VLDL\(C\) was greater after ingestion of sunflower oil than after ingestion of an equivalent amount of beef tallow (Figure 5). The VLDL\(C\) subfraction is thought to contain the majority of hepatic VLDLs (32). Our findings coincide with those of Sakr et al (14), who reported a 3-fold higher triacylglycerol response in chylomicrons after ingestion of a sunflower-oil–rich meal than after ingestion of a beef-tallow–rich meal. Muesing et al (11) also showed a difference in triacylglycerol response in a crossover trial in which men were given emulsions containing 100 g corn oil or beef tallow. Corn oil produced a greater triacylglycerol response in total plasma. In our study, the difference in triacylglycerol response to the test meals was detected in triacylglycerol-rich lipoproteins but not in total plasma, which may reflect the smaller fat load (60 g) administered to our subjects.

The lower lipemic response to the meal containing beef tallow as compared with that containing sunflower oil could be due to lower production of chylomicrons or VLDLs, or more rapid clearance of these particles, which is mediated by lipoprotein lipase (6). Lower production of chylomicrons or VLDLs could result from lower digestibility of beef tallow. In an early study, Mattson (38) showed that the digestibility coefficient of a fat is inversely proportional to its content of tristearin. In a subsequent study, Mattson et al (39) showed that the absorbability of the various triacylglycerols of stearic and oleic acids in rats was directly related to the concentration of stearic acid in the \(sn\)-2 position of the triacylglycerol molecule. More recently, Jones et al (40) found that absorption efficiency for \([1^3\text{C}]\)stearic acid was 78.0% compared with 97.2% and 99.9% for oleic and linoleic acids, respectively, in a study that used stable-isotope-labeled fatty acids. Thus, in stearic acid–rich structures, total fat absorption is adversely affected by the total stearic acid content and correlates with the concentration of stearic acid in...
Apart from differences in digestibility, there could be effects of the fatty acid composition of a meal on molecular processes involved in chylomicron assembly, secretion, and processing. The size, but not the number, of chylomicron particles is greater after ingestion of fats rich in polyunsaturated fatty acids than after fats rich in more saturated fatty acids (14). Larger chylomicron particles may reflect more rapid overall triacylglycerol absorption, and are consistent with the more rapid esterification within the intestinal mucosa of linoleic acid, a polyunsaturated fatty acid, compared with palmitic acid, a saturated fatty acid (46). As hypothesized by Ockner et al (46), different rates of esterification could, in turn, reflect the rates at which polyunsaturated and saturated fatty acids gain access to microsomal esterification enzymes. Polyunsaturated fatty acids have higher affinity than more saturated polyunsaturated fatty acids gain access to microsomal esterification enzymes. Polyunsaturated fatty acids have higher affinity than more saturated fatty acids for fatty acid binding protein (FABP; 46), and therefore are transported more rapidly within the mucosal cell. The larger chylomicrons produced after ingestion of polyunsaturated fatty acids are more rapidly cleared (47–49) because of different susceptibility to lipolytic enzymes (48, 50). We observed greater chylomicronemia after ingestion of sunflower oil, which is not consistent with accelerated catabolism of polyunsaturated fatty acid–rich chylomicrons but is consistent with poor digestibility of beef tallow (14).

The ingestion of cholesterol in the beef tallow does not appear to account for the different lipemic and β-carotene responses to the test meals (Table 2). After a single meal, the majority of cholesteryl esters in chylomicrons originate from endogenous sources rather than dietary cholesterol (51). Addition of ≤140 mg cholesterol to a fat-enriched meal does not alter the postprandial lipoprotein response in healthy subjects (2, 52). After ingestion of the test meals, the changes in cholesterol content from baseline in triacylglycerol-rich lipoproteins, in lipoproteins with density >1.006 kg/L, and in total plasma were not significantly different between the 2 test meals (data not shown). The differences in the apparent intestinal absorption of β-carotene were most likely due to differences in the ingested fatty acids; the sunflower-oil–rich meal was high in linoleic acid, whereas the beef-tallow–rich meal was high in oleic, palmitic, and stearic acids (Table 3).

In rats, Hollander and Ruble (12) showed that addition of linoleic acid (18:2) to an intestinal perfusate resulted in lower rates of intestinal absorption of β-carotene than did addition of oleic acid (18:1). They hypothesized that FABP, which is necessary for the intracellular transport of fatty acids (53), may also function in the intracellular transport of β-carotene. Long-chain polyunsaturated fatty acids, which have greater binding affinity for FABP than do more saturated long-chain fatty acids (46), may more effectively compete with β-carotene for FABP-mediated intracellular transport, resulting in lower intestinal β-carotene absorption. This model is consistent with the β-carotene and triacylglycerol responses to meals of different fatty acid composition that we have now shown in humans. We conclude that ingestion of β-carotene with a sunflower-oil–rich meal high in polyunsaturated fatty acids, as compared with a beef-tallow–rich meal, results in a greater triacylglycerol response and lower apparent absorption of β-carotene, as measured by its appearance in plasma triacylglycerol-rich lipoproteins.

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β-CAROTENE ABSORPTION AFTER FAT-RICH MEALS

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