Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection1–3

Melody J Brown, Mario G Ferruzzi, Minhthy L Nguyen, Dale A Cooper, Alison L Eldridge, Steven J Schwartz, and Wendy S White

ABSTRACT

Background: The amount of dietary fat required for optimal bioavailability of carotenoids in plant matrices is not clearly defined.

Objective: The objective was to quantify the appearance of carotenoids in plasma chylomicrons after subjects ingested fresh vegetable salads with fat-free, reduced-fat, or full-fat salad dressings.

Design: The subjects (n = 7) each consumed 3 salads consisting of equivalent amounts of spinach, romaine lettuce, cherry tomatoes, and carrots with salad dressings containing 0, 6, or 28 g canola oil. The salads were consumed in random order separated by washout periods of ≥2 wk. Blood samples were collected hourly from 0 to 12 h. Chylomicrons were isolated by ultracentrifugation, and carotenoid absorption was analyzed by HPLC with coulometric array detection.

Results: After ingestion of the salads with fat-free salad dressing, the appearance of α-carotene, β-carotene, and lycopene in chylomicrons was negligible. After ingestion of the salads with reduced-fat salad dressing, the appearance of the carotenoids in plasma chylomicrons increased relative to that after ingestion of the salads with fat-free salad dressing (P < 0.04). Similarly, the appearance of the carotenoids in plasma chylomicrons was higher after the ingestion of salads with full-fat than with reduced-fat salad dressing (P < 0.02).

Conclusions: High-sensitivity HPLC with coulometric array detection enabled us to quantify the intestinal absorption of carotenoids ingested from a single vegetable salad. Essentially no absorption of carotenoids was observed when salads with fat-free salad dressing were consumed. A substantially greater absorption of carotenoids was observed when salads were consumed with full-fat than with reduced-fat salad dressing.

KEY WORDS α-Carotene, β-carotene, bioavailability, carotenoids, chylomicrons, coulometric array, electrochemical detection, fat, fat-reduced salad dressings, intestinal absorption, lycopene, salad, salad dressing, vegetables

INTRODUCTION

Bioaccessibility is defined as the fraction of carotenoid transferred during digestion from the food matrix to mixed micelles and thus made accessible for intestinal absorption (1, 2). The bioaccessibility of carotenoids in vegetables is remarkably low. In an in vitro digestion model system, only 1–3% of the β-carotene in raw carrots is accessible for absorption (1, 3); the accessibility of lycopene in canned and fresh tomatoes is <1% (1). Disrupting or weakening fibrous plant cell walls by homogenization or heating substantially increases the release of carotenoids (4–6). Intestinal absorption of carotenoids depends on the presence of fat (7, 8). However, the amount required for optimal bioaccessibility/bioavailability is not clearly defined. Conceivably, the amount of added fat might determine the extent of: 1) release of carotenoids from plant matrices, 2) transfer to the fat phase of the gastrointestinal contents, and 3) solubilization in micelles. Using the in vitro digestion method, addition of 20% cooking oil (% of the dry wt of the carrot sample) to lyophilized raw carrots increased the bioaccessibility of β-carotene by 30% (3). Interestingly, fat was effective only when added to homogenized raw carrots and not to raw carrot pieces. The data indicate an interaction of added fat with the integrity of the plant matrix. There is a need for the study of such interactions in humans.

The adoption of a low-fat diet has been associated with decreased risks of chronic disease and obesity (9). The use of fat-modified foods can be a successful strategy for decreasing fat intake (10, 11). Using data from the 1985 Continuing Survey of Food Intakes by Individuals, Krebs-Smith et al (12) identified salad dressings as the major source of fat in women’s diets. The food industry has targeted such key sources of fat in the diet to provide fat-modified options. Over the past 10 y, there has been steady growth in demand for low-fat salad dressings and mayonnaise (13). In the United States, 20% of men and 33% of women report that they always choose low-calorie instead of regular salad dressings (14). The vegetables in salads are essentially fat-free but rich in carotenoids, which need fat to be absorbed. By choosing reduced-fat or fat-free salad dressings, consumers could potentially compromise their exposure to the

1 From the Department of Food Science and Human Nutrition and the Center forDesigning Foods to Improve Nutrition, Iowa State University, Ames (MJB and WSW); the Department of Food Science and Technology, The Ohio State University, Columbus (MGF, MLN, and SJ); and The Procter & Gamble Nutrition Science Institute, Cincinnati (DAC and ALE).
2 Supported by The Procter & Gamble Nutrition Science Institute, Cincinnati; the Hatch Act; and the State of Iowa.
3 Reprints not available. Address correspondence to WS White, Department of Food Science and Human Nutrition, 1111 Human Nutritional Sciences Building, Iowa State University, Ames, IA 50011-1120. E-mail: wswine@iastate.edu.

Received July 31, 2003.
Accepted for publication February 12, 2004.
putative bioactivity of those carotenoids in preventing heart disease (15, 16), cancer (17), and other chronic diseases (18).

The objective of this study was to use HPLC with coulometric array electrochemical detection (ECD) to compare carotenoid absorption from a salad of mixed fresh vegetables ingested with salad dressing containing 0, 6, or 28 g fat. Ferruzzi et al (19) developed protocols to measure carotenoids in microsamples of human tissue and plasma by using HPLC-ECD. They found ECD to be 100–1000 times more sensitive than conventional ultraviolet light visible (UV/VIS) detection. We hypothesized that HPLC-ECD could be applied to reliably measure nanomolar amounts of carotenoids absorbed from a single mixed-vegetable salad.

SUBJECTS AND METHODS

Subjects

Ten healthy, nonsmoking, normolipidemic men and women 19–28 y of age were enrolled in the study. Two of the women were found to have veins incompatible with phlebotomy and were dropped from the study before completing the first study period. A male subject who completed the study was later found to have high carotenoid and triacylglycerol concentrations in the plasma chylomicron fraction collected at baseline after a 12-h (overnight) fast and immediately before ingestion of the test salad. We suspected noncompliance and therefore excluded the data from this subject. Data from the remaining 3 men and 4 women were included in our analyses.

The subjects were screened initially by interview with the use of a standardized questionnaire that addressed health and lifestyle factors. At the time of the interview, anthropometric data (height and weight) were also collected. Those who qualified for the study on the basis of the eligibility criteria then underwent a physical examination, pregnancy test (female subjects), complete blood count, and blood chemistry profile, which included the measurements of blood lipids, serum thyroxine, and thyroid-stimulating hormone. The exclusion criteria 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 might affect lipid absorption or transport (including antibiotics), current or recent (previous 6 mo) use of dietary supplements, frequent consumption of alcoholic beverages (>1 drink/d), hypothyroidism or hyperthyroidism on the basis of serum thyroxine and thyroid-stimulating hormone concentrations, and hyperlipidemia on the basis of the plasma lipid and lipoprotein profile. Vegetarians and those with a history of anemia or excessive bleeding, a psychological aversion to phlebotomy, chronic disease, lipid malabsorption or intestinal disorders, photosensitivity disorders, menstrual cycle disorders, eating disorders, lactose intolerance, unusual food habits (such as extreme weight loss diets, avoidance of a food group), or a recent change in weight (>4.5 kg in previous 1 mo) were also excluded. 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

The subjects were provided a list of carotenoid-rich foods and instructed to avoid those foods for 4 d preceding each of the 3 study periods. On the day preceding consumption of the test salad, the subjects consumed a controlled, low-carotenoid diet of conventional foods. The macronutrient content of the diet was analyzed by NUTRITIONIST V software (N-Squared Computing Inc, Salem, OR). The diet provided 9.29 and 12.02 MJ, 84 and 107 g protein (15% of energy), 66 and 90 g fat (27% of energy), and 329 and 417 g carbohydrate (58% of energy) for the female and male subjects, respectively. The next morning, each subject consumed the test salad followed by a low-fat lunch and dinner, which were identical for male and female subjects. The lunch provided 2.22 MJ, 30.0 g protein, 5.5 g fat, and 88.0 g carbohydrate. The dinner provided 2.11 MJ, 37.5 g protein, 5.7 g fat, and 75.5 g carbohydrate. All foods were weighed, prepared, and consumed under supervision in the Human Metabolic Unit of the Center for Designing Foods to Improve Nutrition, Iowa State University. The exceptions were the lunch, afternoon, and evening snacks on the day preceding the test meal, which were prepared in advance and carried out. The carotenoid content in duplicate aliquots of composites of the diet was analyzed by HPLC according to the protocol used by White et al (20). The controlled low-carotenoid diet on the day preceding the test meal provided no detectable α-carotene or lycopene; the diet of the male subjects contained 15 μg β-carotene, 290 μg lutein, and 58 μg zeaxanthin, whereas the diet of the female subjects contained 15 μg β-carotene, 217 μg lutein, and 50 μg zeaxanthin. Together, the 2 low-fat meals given to both the male and female subjects on the day of test salad consumption contained a total of 28 μg β-carotene, 83 μg lutein, 34 μg zeaxanthin and no detectable α-carotene or lycopene.

Test salads

The test salad consisted of 48 g fresh spinach (Popeye Flat Leaf Spinach; River Ranch Fresh Foods, Salinas, CA), 48 g romaine lettuce (Romaino Prepackaged Salad Mix; Dole, Salinas, CA), 66 g raw shredded carrots (Dole Prepackaged Shredded Carrots; Dole), and 85 g raw cherry tomatoes (Capitol City Fruit Co, Norwalk, IA) (~6.5 cherry tomatoes). The combined weight (247 g) was chosen as the 90th percentile of the quantity of salad (lettuce and other vegetables) eaten by adults per eating occasion in the 1994–1996 Continuing Survey of Food Intakes by Individuals (21). The Romaino Prepackaged Salad Mix was sorted to exclude radicchio and to include only romaine lettuce. To minimize variation in the carotenoid content of the salads, romaine and spinach leaves were each manually sorted to provide uniform green color, which was used as an indicator of chlorophyll and, by extrapolation, carotenoid content. The biosynthesis of chlorophyll and carotenoids is quantitatively coordinated in chloroplasts (22). The salad dressing was prepared from a commercial salad dressing mix containing dry ingredients (Good Seasons Italian Dressing Mix; Kraft Foods, Glenview, IL). To prepare the salad dressings, 59 g white vinegar (HJ Heinz Co, Pittsburgh) and 44 g water were added to the dry mix along with canola oil (Hunt-Wesson Inc, Fullerton, CA) or additional water substituted on a weight-by-weight basis to create full-fat (112 g canola oil), reduced-fat (24 g canola oil, 85 g water), and fat-free (112 g water) salad dressings. The fat contents of the prepared salad dressings were analyzed in duplicate by Covance Laboratories Inc (Madison, WI) by using total fat acid hydrolysis (23). A 60-g serving of the fat-free, reduced-fat, or full-fat salad dressings contained 0, 6, or 28 g fat, respectively. The serving size and corresponding fat contents of the salad dressings were chosen to...
approximate the 90th percentile of the quantity of low-calorie and full-fat salad dressings reported by adults eating salad components at an eating occasion in the 1994–1996 Continuing Survey of Food Intakes by Individuals (21). The intent was to simulate the typical intake of salad dressing for a person eating a salad as all or most of a meal.

The salad and salad dressing were served in separate bowls. The subjects were given a spathula and instructed to scrape the salad dressing out of the bowl and onto the salad. Finally, subjects were instructed to wipe the dish with a lettuce leaf to ensure all of the salad dressing was ingested. The salad was served with 500 mL water.

Experimental design

Each subject ingested 3 test salads that had identical vegetable compositions but that had Italian salad dressings with different fat contents (0, 6, or 28 g canola oil). The 3 salad dressings were ingested in random order with the salads, each separated by washout periods of ≥2 wk.

Blood samples were collected by syringe via a catheter with a disposable obturator that had been placed in a forearm vein by a registered nurse. A baseline 12-mL blood sample was drawn in the morning after an overnight (12-h) fast and immediately preceding ingestion of the test salad. Additional 12-mL blood samples were collected at hourly intervals for 12 h after ingestion of the test salad. Blood was transferred to EDTA-containing tubes and immediately placed on ice, protected from light, and centrifuged (1380 × g, 4 °C, 30 min) to separate plasma. After each blood sample was drawn, 3 mL sterile physiologic saline (9 g NaCl/L) was injected into the catheter via the obturator to prevent clotting and then withdrawn immediately before the next blood sample was drawn. The subjects were fed the low-carotenoid, low-fat (5.7 g fat) lunch after the 5th hourly blood sample was drawn and the low-carotenoid, low-fat (5.7 g fat) supper after the 10th blood sample was drawn.

Chylomicron isolation

Chylomicrons were used as a vehicle to distinguish newly absorbed from endogenous carotenoids (24). Cumulative rate ultracentrifugation (25–27) was used to isolate the chylomicron fraction with high purity and high recovery, as reflected by the absence of apolipoprotein B-48 in higher-density fractions (28).

Salt solutions were prepared (1.006, 1.065, and 1.020 g/mL) by using sodium chloride, potassium bromide, and sodium EDTA to form a density gradient. The densities were confirmed by using a digital density meter (DMA-48, Anton-PAAR USA, Ashland, VA). Plasma was transferred to a centrifuge tube (Ultra Clear; Beckman Instruments, Inc, Spincvo Division, Palo Alto, CA), and the density was adjusted to 1.10 kg/L by adding potassium bromide. 4-(Chloromercuri)benzenesulfonic acid was added as a preservative. The plasma was overlaid with the prepared density solutions in order of most to least density. Plasma samples were centrifuged in an ultracentrifuge (L8-70 M; Beckman Instruments Inc, Palo Alto, CA) with the use of a swinging bucket rotor (SW 40/) at 28.3K for 43 min. The chylomicron fraction was removed from the centrifuge tube and stored at −80 °C until analyzed. The procedures were carried out under yellow light.

Extraction and HPLC-ECD analysis of carotenoids in the chylomicron fraction

Carotenoids were extracted from 2.0-mL aliquots of the plasma chylomicron fraction by deproteinization with ethanol and 3 extractions with hexane containing 1.0 g butylated hydroxytoluene/L. The combined hexane layers were dried under nitrogen, redissolved in 50:50 (by vol) methanol:methyl-tert-butylether (MTBE), and analyzed immediately. The carotenoids were separated by HPLC with a C18 Carotenoid Column (Waters, Milford, MA). The components included a Hewlett-Packard (Wilmington, DE) model 1050 pump, autosampler, and solvent prep station. The pump was a quaternary gradient-capable system, and the solvents were degassed by helium sparge. A gradient elution system that consisted of different proportions of ethanol:water:MTBE:1.0 M ammonium acetate buffer (pH 4.5) in reservoirs A (88:5:5:2) and B (25:0:73:2) was used. Initial conditions were 100% solvent A, which were followed by a linear gradient to 15% solvent A and 85% solvent B from 0 to 40 min. The flow rate was 1 mL/min. An ESA model 5600 Coularray electrochemical detector (Chelmsford, MA) with 8 channels in series was used for the analysis (19). The potential of the 8 channels was set from 200 to 680 mV in 60-mV increments; the major carotenoids responded dominantly between 380 and 500 mV. The ESA COULARRAY software and data management system were used to collect and integrate the chromatographic data.

Extraction and HPLC analysis of carotenoids in salad vegetables

Representative samples of spinach, romaine lettuce, cherry tomatoes, and carrots from each of the 3 study periods were stored at −70 °C and analyzed in duplicate according to a modification of the method of Hart and Scott (29). Vegetable tissue was processed in a food processor (Handy Chopper Plus HC3000; Black & Decker Corp, Towson, MD). A 10-g sample of processed vegetable tissue was combined with 4 g celite, 1 g solid CaCO3 to neutralize organic acids, and 50 mL methanol and tetrahydrofuran (1:1, by vol) containing 0.1 g butylated hydroxytoluene/L and ethyl β-apo-8’-carotenoate (Fluka, Milwaukee) as an internal standard. Carotenoids were extracted from the plant tissue by homogenizing for 1 min with a Brinkman (Westbury, NY) homogenizer. The resulting suspension was filtered through no. 1 and 42 Whatman filter papers in a Buchner funnel under vacuum (30). The filter cake was resuspended with 50 mL methanol and tetrahydrofuran (1:1, by vol), homogenized for 1 min, and filtered through the same filter papers. The extraction of the filter cake with methanol and tetrahydrofuran was repeated.

The combined methanol and tetrahydrofuran filtrates were transferred to a separatory funnel. The carotenoids were extracted by adding 50 mL petroleum ether (boiling range: 41.5–56.5 °C, containing 0.1 g butylated hydroxytoluene/L) and 50 mL NaCl solution (100 g/L), which was followed by careful shaking (30). The lower aqueous-methanol-tetrahydrofuran phase was collected, and the upper petroleum ether phase was transferred to a 200-mL volumetric flask. The aqueous-methanol-tetrahydrofuran phase was extracted 2 more times with 50-mL aliquots of petroleum ether. The petroleum ether layers were transferred to the same 200-mL volumetric flask, which was then brought to volume with additional petroleum ether. For cherry tomatoes, a 4-mL aliquot was evaporated to dryness under
CAROTENOID ABSORPTION FROM VEGETABLE SALADS

Analysis of the triacylglycerol content of the chylomicron fraction

A GPO-Trinder triacylglycerol diagnostic kit (reagent 339; Sigma) was modified for use with a microplate reader as follows. The GPO-Trinder reagent was reconstituted with 25% of the underreconstituted GPO-Trinder reagent. After a 45-min incubation at room temperature, samples were analyzed with a microplate reader (Elx 808; Bio-Tek Instruments Inc, Winooski, VT), which agitated the microplate to mix the contents and then measured the absorbance of the color change reaction at 562 nm. The system operated with KC Junior software version 1.14, 1998 (Bio-Tek Instruments Inc). The content of zeaxanthin in photosynthetic tissues is dependent on light exposure during growth (32).

Statistical analyses

Blood samples were collected for 12 h after ingestion of the test salad. Because the chylomicron carotenoid contents returned to baseline values by 9 h, only data for 0–9 h were used to compare treatments. Similarly, chylomicron triacylglycerol contents returned to baseline by 8 h; thus, only data for 0–8 h were used in statistical analyses. Statistical analyses were performed by using SAS (version 8.2; SAS Institute Inc, Cary, NC). The plasma chylomicron carotenoid and triacylglycerol data were analyzed by using repeated-measures analysis of variance (ANOVA). When the P value obtained was significant, treatments were compared by using one-sided Tukey’s multiple comparison tests. A P value < 0.05 was considered significant.

RESULTS

Subject characteristics

The average age of the 3 male and 4 female subjects was 22 ± 1.1 y (range: 19–28 y). The subjects’ average body mass index (in kg/m²) was 23.4 ± 1.5 (range: 19.1–29.0). The subjects were in good health on the basis of the exclusionary criteria previously described, physical examination, and blood biochemistry profile.

Carotenoid composition of the test salad

The total carotenoid content of the test salad (α-carotene, β-carotene, lutein, lycopene, and zeaxanthin) was 31.3 mg. Only α-carotene, β-carotene, and lycopene were measured in plasma chylomicrons; of these, α-carotene had the lowest content (4.4 mg) in the salad (Table 1). The measured carotenoid contents in the individual vegetables were similar to those reported in the USDA–Nutrition Coordinating Center Carotenoid Database for US Foods—1998 (31). The exception was cherry tomatoes, which had a higher lycopene content than the reported values, which were for regular tomatoes. In studies of carotenoid absorption, fresh vegetables are infrequently used because their carotenoid composition can vary widely. In the current study, we took measures to ensure a consistent carotenoid content of the test salads. Romaine lettuce and spinach were visually sorted to provide greater uniformity of the color of the leaves, and, consequently, of the carotenoid content. Each of the vegetables was purchased prepackaged by a single company. Zeaxanthin (found in the romaine lettuce and spinach) had the most variable content. The content of zeaxanthin in photosynthetic tissues is dependent on light exposure during growth (32).

Change in chylomicron carotenoid content after ingestion of the test salad

Carotenoid absorption from the test salad was readily detected and quantified in the postprandial chylomicron fraction by using vacuum; the dried lipid extract was reconstituted in 1 mL MTBE followed by 1 mL methanol. For the other 3 vegetables, a 10-mL aliquot was evaporated to dryness under vacuum, and the dried lipid extract was reconstituted with 400 µL MTBE and 1600 µL methanol. The reconstituted extract was filtered through a 0.2-µm nylon syringe filter (Alltech, Deerfield, IL), and a 25-µL aliquot was injected into the HPLC system. The components included a 717 Plus autosampler with the temperature control set at 5 °C, two 510 solvent-delivery systems, and a 996 photodiode array detector (Waters Corporation, Milford, MA). The system included a 717 Plus autosampler with the temperature control set at 5 °C, two 510 solvent-delivery systems, and a 996 photodiode array detector (Waters Corporation, Milford, MA). The system operated with Millennium^® Software version 3.05.01 (Waters Corporation). Carotenoids were separated on a 5-µm C₃₀ Carotenoid Column (4.6 × 250 mm; Waters Corporation) eluted by using a linear mobile-phase gradient from 100% methanol (containing 1 g ammonium acetate/L) to 100% MTBE over 55 min. The flow rate was 2.0 mL/min. Solvents were HPLC grade; the methanol, MTBE, and ammonium acetate were purchased from Fisher Scientific (Chicago). Petroleum ether and tetrahydrofuran were purchased from VWR (Boston). Internal standard calibration curves were generated for α-carotene (Carolina Chemical Purities, Cary, NC), β-carotene (Fluka), β-carotene, lutein (Kemin Foods, Des Moines), lycopene (Sigma, St Louis), and zeaxanthin (Indofine Chemicals, Belle Mead, NJ).

### Table 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight</th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>Lutein</th>
<th>Lycopene</th>
<th>Zeaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot, grated</td>
<td>66</td>
<td>4.444 ± 0.388²</td>
<td>6.956 ± 0.343</td>
<td>0.050 ± 0.006</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lettuce, romaine</td>
<td>48</td>
<td>0</td>
<td>1.639 ± 0.236</td>
<td>1.650 ± 0.181</td>
<td>0</td>
<td>0.052 ± 0.005</td>
</tr>
<tr>
<td>Spinach, leaf</td>
<td>48</td>
<td>0</td>
<td>2.830 ± 0.196</td>
<td>0.060 ± 0.264</td>
<td>0</td>
<td>0.111 ± 0.024</td>
</tr>
<tr>
<td>Tomato, cherry</td>
<td>85</td>
<td>0</td>
<td>0.799 ± 0.101</td>
<td>0.115 ± 0.011</td>
<td>8.613 ± 0.293</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>247</td>
<td>4.444 ± 0.388</td>
<td>12.224 ± 0.038</td>
<td>5.875 ± 0.444</td>
<td>8.613 ± 0.293</td>
<td>0.163 ± 0.024</td>
</tr>
</tbody>
</table>

¹n = 3. A representative sample of each vegetable from each of the 3 study periods was analyzed in duplicate.

²x ± SEM (all such values).
HPLC-ECD (Figure 1). Several cis isomers of lycopene were detected but not quantified. Lutein and zeaxanthin were not measured because they co-eluted with the high content of tocopherol in the plasma chylomicron fraction resulting from ingestion of canola oil in the salad dressing.

When the salads were ingested with fat-free salad dressing, the absorption of carotenoids was negligible (Figure 2). When the salads were ingested with reduced-fat salad dressing, the added fat promoted α-carotene, β-carotene, and lycopene absorption. Similarly, there was substantially more absorption of the carotenoids when salads were consumed with full-fat than with reduced-fat salad dressing. Absorption of lycopene from the cherry tomatoes was disproportionately low compared with the relative contents and absorption of α-carotene in the carrots or total β-carotene contributed by all of the salad vegetables.

Change in chylomicron triacylglycerol content after ingestion of the test salad

After consumption of the test salad with fat-free salad dressing, there was a negligible appearance of triacylglycerol in chylomicrons relative to baseline, which was similar to the response of the carotenoids (Figure 3). When the salad was ingested with reduced-fat salad dressing, more triacylglycerol appeared in the chylomicron fraction. Similarly, there was substantially more absorption of triacylglycerol when salads were consumed with full-fat than with reduced-fat salad dressing.

The peak triacylglycerol content in chylomicrons occurred 3 h after consumption of the test salad with reduced-fat or full-fat salad dressings, with a smaller peak at 6 h (Figure 3). A lunch containing 5.5 g fat was given after the 5-h blood draw. The second triacylglycerol peak at 6 h likely corresponded to triacylglycerol released from the intestine during the postprandial period. The appearance of carotenoids in chylomicrons peaked 3 and 4 h after ingestion of the test salad with reduced-fat and full-fat salad dressings, respectively (Figure 2). A second smaller peak in chylomicron carotenoid contents was observed at 6 h, which coincided with the second triacylglycerol peak.

DISCUSSION

We applied the high sensitivity of coulometric array ECD to measure the appearance of carotenoids in the plasma chylomicron fraction of subjects who ingested a single serving of fresh vegetables. Plasma chylomicrons were used as a vehicle to isolate newly absorbed carotenoids appearing in total plasma (24). Using HPLC with UV/VIS detection, previous investigators were unable to reliably detect carotenoids in total plasma (33) or in the chylomicron fraction (34) after a single serving of fresh vegetables was ingested. They concluded that a more sensitive approach was needed to enable measurement of the low amounts of carotenoids absorbed from plant matrices (34). Other investigators overcame the sensitivity constraint of UV/VIS detection by having subjects ingest large servings of fresh vegetables, such as tomatoes or more realistic servings of relatively bioaccessible carotenoids in...
Carotenoids are most commonly consumed in fruit and vegetables, and most populations depend on provitamin A carotenoids in these foods to meet vitamin A requirements. Interactions of dietary fat with carotenoids ingested within plant matrices are thus particularly relevant. In plants, carotenoids are compartmentalized within plastids (chloroplasts and chromoplasts), which, in turn, are contained within fibrous cell walls. Disruption of the plant matrix (via homogenization or heating) promotes release and absorption of carotenoids (6). Dietary fat may be particularly essential to the bioaccessibility of fat-soluble carotenoids in vegetables to promote their release from the plasma triacylglycerol-rich lipoprotein fraction (density $1.006 \, \text{kg/L}$) during digestion and thereby made accessible for absorption. Dietary fiber, particularly soluble fiber such as pectin, interferes with the absorption of $\beta$-carotene (38) and with the lipolysis and the assimilation of lipids (39). The degree of micellar solubilization of vegetable carotenoids in the duodenum is related to the efficiency of triacylglycerol hydrolysis (2). Fibrous plant matrices are likely to interfere with lipolysis and the micellar solubilization of carotenoids; an effect that potentially could be minimized by ingestion with more fat. In our study, the bioavailability of lycopene ingested in tomatoes was particularly low, which is consistent with previous reports (2, 40). Lycopene bioavailability showed the most dramatic improvement when added fat was increased from 6 to 28 g. The pronounced fat effect for lycopene is consistent with the hypothesized interaction of fat and the extent of micellar solubilization of vegetable carotenoids.

Bioefficacy is defined as the efficiency with which an ingested provitamin A carotenoid is absorbed and converted to vitamin A (41). In our study, no effort was made to quantify retinyl ester cleavage products. However, stable tracer studies in humans suggest that absorption of intact $\beta$-carotene is a good predictor of bioefficacy (42). To date, studies in vitamin A–deficient subjects have produced equivocal results regarding the amount of fat needed to optimize the bioefficacy of $\beta$-carotene in plant matrices. Jalal et al (43) found that the amount of added fat needed for optimal bioefficacy of $\beta$-carotene exceeded 4 g per meal when red sweet potatoes were fed to vitamin A–deficient Indonesian children. In an early study, Jayarajan et al (44) reported no difference in serum retinol concentrations in Indonesian children who ingested 40 g spinach curry with either 5 or 10 g groundnut oil. The treatment groups were not matched according to the baseline serum retinol concentrations in the children. Only 8 of 22 children ingesting 10 g fat were vitamin A deficient, as indicated by serum retinol concentrations $\leq 0.70 \, \mu\text{mol/L}$, whereas 14 of 22 children ingesting 5 g fat had such low serum retinol concentrations. The latter group would be expected to be more responsive to dietary intervention. Takyi (45) reported similar improvements in serum retinol in vitamin A–deficient children fed dark green leafy vegetables in stews containing either 1.3 or 5.1 g fat. At baseline, 20% of the children had worm infestations. Previously, Jalal et al (43) found that added fat enhanced the bioefficacy of $\beta$-carotene in red sweet potato only in children who did not have substantial Ascariis infestation. In children with high egg counts, the bioefficacy of $\beta$-carotene was equivalent in the absence or presence of added fat. The findings of studies suggest that adding fat to the diets of populations low in dietary fat and vitamin A can improve vitamin A status without intervention to change carotenoid or vitamin A intakes (46, 47).
The threshold of 3–5 g fat per meal reported by Roodenburg et al. (37) is being adopted as a guideline to promote optimal absorption of β-carotene (48). Our data suggest that 3–5 g fat should be considered a minimal amount that may not be sufficient for optimal bioaccessibility of carotenoids within the intact structures of plants. We showed that use of fat-free or reduced-fat salad dressings may still afford consumers optimal access to the putative health benefits of carotenoids while promoting a diet moderate in total fat, as recommended by US Dietary Guidelines for Americans (49). The effect of low-fat diets on the carotenoid status of free-living populations is a current research focus (50).

We thank Lynn Lanning and Gretchen Zitterich for implementing the phlebotomy protocols, John Conner (Sigma-Aldrich) for technical assistance with the modification of the Sigma GPO-Trinder triacylglycerol diagnostic kit (reagent 339) for use with a microplate reader, and Ivan Ramlar for assistance with the statistical analyses.

WSW and DAC designed the study. ALE designed the specific components of the test salads based on national food-consumption data. MJB implemented the study protocol, isolated plasma chylomicrons, and completed chylomicron triacylglycerol and vegetable carotenoid analyses under the direction of WSW. MGF and MLN completed the carotenoid analyses in the plasma chylomicron fractions using a method developed by SJS. MJB and WSW completed the statistical analyses and wrote the manuscript. ALE and DAC were employed by The Procter & Gamble Co., which funded the research. None of the other authors had a financial interest in The Procter & Gamble Co.

REFERENCES

6. van het Hof KH, de Boer BCJ, Tijburg LBM, et al. Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. J Nutr 2000;130:1189–96.


