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Tyssandier, Viviane, Emmanuelle Reboul, Jean-François Dumas, Corinne Bouteloup-Demange, Martine Armand, Julie Marcand, Marcel Sallas, and Patrick Borel. Processing of vegetable-borne carotenoids in the human stomach and duodenum. *Am J Physiol Gastrointest Liver Physiol* 284: G913–G923, 2003. First published January 10, 2003; 10.1152/ajpgi.00410.2002.—Carotenoids are thought to diminish the incidence of certain degenerative diseases, but the mechanisms involved in their intestinal absorption are poorly understood. Our aim was to obtain basic data on the fate of carotenoids in the human stomach and duodenum. Ten healthy men were intragastrically fed three liquid test meals differing only in the vegetable added 3 wk apart and in a random order. They contained 40 g sunflower oil and mashed vegetables as the sole source of carotenoids. Tomato purée provided 10 mg lycopene as the main carotenoid, chopped spinach (10 mg lutein), and carrot purée (10 mg β-carotene). Samples of stomach and duodenal contents and blood samples were collected at regular time intervals after meal intake. all-*trans* and *cis* carotenoids were assayed in stomach and duodenal contents, in the fat and aqueous phases of those contents, and in chylomicrons. The *cis-trans* β-carotene and lycopene ratios did not significantly vary in the stomach during digestion. Carotenoids were recovered in the fat phase present in the stomach during digestion. The proportion of all-*trans* carotenoids found in the micellar phase of the duodenum was as follows (means ± SE): lutein (5.6 ± 0.4%), β-carotene (4.7 ± 0.3%), lycopene (2.0 ± 0.2%). The proportion of 13-*cis* β-carotene in the micellar phase was significantly higher (14.8 ± 1.6%) than that of the all-*trans* isomer (4.7 ± 0.3%). There was no significant variation in chylomicron lycopene after the tomato meal, whereas there was significant increase in chylomicron β-carotene and lutein after the carrot and the spinach meals, respectively. There is no significant *cis-trans* isomerization of β-carotene and lycopene in the human stomach. The stomach initiates the transfer of carotenoids from the vegetable matrix to the fat phase of the meal. Lycopene is less efficiently transferred to micelles than β-carotene and lutein. The very small transfer of carotenoids from their vegetable matrices to micelles explains the poor bioavailability of these phytomicroconstituents.

β-carotene; lycopene; lutein; postprandial; absorption; bioavailability

EPIDEMIOLOGICAL STUDIES CONSISTENTLY associate diets rich in fruits and vegetables with a lower incidence of several diseases (22). The plant pigments, carotenoids, are assumed to be involved in this effect because of their antioxidant properties (20, 21).

In common foods, carotenoids are mostly found as all-*trans* isomers (25), but significant amounts of geometrical *cis* isomers can be produced during processing (36, 37). The *cis* isomers of β-carotene and lycopene have attracted attention, because several studies have suggested that their bioavailabilities are different from those of their corresponding *trans* isomers (2, 15, 18, 39) and that they may possess specific functions (16, 23, 26, 28).

The absorption efficiency of all-*trans* β-carotene, the most extensively studied carotenoid, is generally poor but widely variable (4, 11), ranging between 3.5 and 90%, depending on the dose, the matrix in which it is incorporated, and the method used to estimate absorption (8, 12, 31, 47). Data on the absorption efficiency of other carotenoids are scant, although such information is needed for dietary recommendations, supplement formulation, and the design of intervention studies involving carotenoids (33). Although a number of factors is thought to affect the bioavailability of carotenoids (46), much work remains to be done to identify the main factors among all those proposed. The mechanisms that might explain the low and variable absorption of carotenoids are largely unknown, because the fate of these micronutrients in the human upper gastrointestinal (GI) tract is still obscure. Three assumptions are currently made about carotenoid metabolism in the lumen of the human upper GI tract. First, carotenoids cannot be absorbed while they remain embedded in their original vegetable matrices. Second, carotenoids have to be solubilized in mixed micelles to

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be absorbed. Third, carotenoids are absorbed by passive diffusion [as suggested for β-carotene (17)]. These assumptions are not sufficient to understand the factors limiting the absorption of natural carotenoids. Further data are required on the role of the stomach in carotenoid absorption, possible *cis-trans* isomerization of carotenoids in the acidic environment of the stomach, the extent of release of carotenoids from their vegetable matrices, and the relative availabilities for absorption of *cis* and *trans* isomers of carotenoids.

The aim of this study was to ascertain the fate of the main natural human dietary carotenoids, i.e., β-carotene, lycopene, and lutein in their natural vegetable matrices, in the lumen of the human upper GI tract, and to determine whether this fate affects their bioavailability. For this purpose, we adapted a model, previously used by us to study lipids (1) and vitamins A and E (5) metabolism in the human GI tract, to study the metabolism of carotenoids supplied in their natural vegetable matrices.

MATERIALS AND METHODS

Subjects. Ten healthy male volunteers (24.2 ± 1.0 yr, 1.80 ± 0.02 m, and 74.7 ± 3.5 kg) were enrolled in the study after giving their written, informed consent. The study was approved by the Regional Ethical Committee on Human Experimentation of Auvergne (France). No volunteer had any history of GI disease or lipid metabolic disorders according to clinical examination, disease history, and fasting plasma lipid parameters [plasma total triacylglycerols (0.53 ± 0.03 mM) and plasma total cholesterol (3.80 ± 0.24 mM) concentrations were in the normal range]. No volunteer was taking any drugs known to affect GI function or lipid metabolism.

The subjects' usual diet was monitored by means of a 5-day food recall. This dietary recall was analyzed for nutrient composition using diet analysis software (GENI; Micro 6, Nancy, France) completed for carotenoids by a carotenoid food-composition database (6). The subjects consumed a typical Western diet, with 11.88 ± 0.90 MJ/day, 14.52 ± 0.78% of energy as proteins, 33.82 ± 1.90% as fat, 48.03 ± 2.58% as carbohydrates, and 3.63 ± 1.45% as alcohol. Carotenoid intake was as follows (mg/day): β-carotene (3.8 ± 0.8), lycopene (2.1 ± 0.5), lutein-zeaxanthin (1.5 ± 0.5), α-carotene (0.9 ± 0.3), and β-cryptoxanthin (0.1 ± 0.04).

Test meals. Each subject was given three test meals differing only in the vegetable added, 3 wk apart and in a random order. The test meal compositions are given in Table 1. Note that vegetables were the sole source of carotenoids. Tomato purée was used as the source of lycopene, carrot purée as the source of β-carotene, and chopped spinach as the source of lutein.

The amount of triacylglycerols incorporated in the meals (40 g) was designed to be in the range of usual human food intake. The meal components were always mixed in the same conditions: 30 s with an electric food blender (Vitamix, Seb, Paris, France) set at 1,500 rpm for 15 min at +4°C to homogenize the components and prepare a lipid emulsion. The amount of carotenoids provided by the meals (~10 mg of the main carotenoid per meal) was slightly higher than that usually consumed by the subjects (see above) but was designed to accurately follow the fate of these micronutrients in the GI tract and in the chylomicron fraction. Note that the vegetables did not in compulsory contain only one type of carotenoid. Indeed, carrot purée provided 10 mg β-carotene and 3.9 mg α-carotene. Tomato purée provided 10 mg lycopene and 1 mg β-carotene. Chopped spinach provided 10 mg lutein, 7.1 mg β-carotene, and 0.3 mg zeaxanthin.

Table 1. *Composition of test meals (g)*

Sunflower oil*	40.0
Cow whey proteins†	52.0
Sucrose	116.0
Soy lecithins‡	5.0
Vegetables§	
Tomato purée (lycopene)	33.6
Carrot purée (β-carotene)	161.0
Chopped spinach (lutein)	99.0

*Unilever Health Institute, Unilever Research, Vlaardingen, Netherlands. †Lacprodan DI-9212, Arla Foods, Skanderborgvej, Denmark. ‡Lécithine 100, Gerblé, Nutrition & Santé, Revel, France. §These amounts of vegetable were chosen to provide similar amounts of carotenoids ≈ 10 mg of the main carotenoid). Vegetable sources were provided by Institute of Food Research (Norwich, UK). NaCl (0.9%) was added to bring the volume to 600 ml. Carrots were peeled, diced, cooked, and pureed with the addition of 50% water to make the puree workable. Fresh spinach leaves were harvested and cooked on the same day. The harvest was split, and one-half was kept as whole leaf and the other half was chopped. The tomato meal provided 4.51 MJ (37.2% as fat, 43.4% as carbohydrates, and 19.4% as proteins). The carrot meal provided 4.65 MJ (36.2% as fat, 44.5% as carbohydrates, and 19.3% as proteins). The spinach meal provided 4.54 MJ (37.1% as fat, 42.9% as carbohydrates, and 20.0% as proteins).

Study design. Each procedure started at 07:30 AM after the subjects had fasted overnight for 12 h. As previously described (1, 5), each subject was intubated with a single-lumen nasogastric tube (16 Fr, 122 cm, Sherwood Medical Argyle, Tullamore, Ireland) and a single-lumen nasoduodenal tube (duodenographie bilboa-dotter set, 12 Fr, 145 cm, William Cook Europe, Bjaeverkov, Denmark). The stomach tube was located 45–50 cm from the nose in the stomach at the corpus-antrum junction. The duodenal tube was located at the junction of the second and the third portions of the duodenum. The position of the tubes was checked radiographically before meal intake. After the tubes were fitted, the volunteers adopted a sitting position that they maintained until the end of the study to limit variations in gastric emptying rates (19). An antecubital vein was catheterized with an intravenous cannula equipped with disposable obturators (Terumo Europe, Leuven, Belgium).

Fasting stomach juice and duodenal fluid were removed by manual aspiration just before ingestion of the liquid test meal. The 600-ml liquid test meal was fed intragastrically by using a 60 ml-syringe over a 20-min period. A 1-ml sample of the initial liquid test meal was kept for measuring emulsion droplet size. Large samples (100–200 ml) of the stomach contents were aspirated at 20, 40, 60, 90, 120, 150, and 180 min after meal intake by gentle aspiration with a 60-ml syringe to obtain representative samples. A 20-ml aliquot was taken from each sample for analytical determinations. The remaining sample was promptly reinjected into the subject's stomach via the nasogastric tube. Duodenal contents (5–10 ml) were aspirated at the same postprandial times as above by using the nasoduodenal tube. Portions (2 ml) of the stomach content sample and portions (0.5 ml) of the duodenal content sample were placed in glass tubes containing double volumes of chloroform/methanol [2:1 (vol/vol)] to stop lipolysis and subsequently analyze lipids. Portions (0.5 ml) of the sample were placed in tubes and stored at 4°C until the size of emulsion lipid droplets was determined (see *Size of emul-*

sified lipid droplets). The remaining fractions of the samples were placed in another tube and stored at −80°C until phase separation and carotenoid analysis (see *Carotenoid analyses*).

A baseline fasting blood sample (0 h) was collected. Blood samples (14 ml) were drawn every hour for 8 h after test meal intake. Plasma was separated from whole blood by centrifuging (910 *g*, 15 min, 4°C). Chylomicron plus large chylomicron remnants (Svenberg floatation unit > 1,000) were isolated by ultracentrifuge as previously described (9).

Size of emulsified lipid droplets. The median size of emulsion lipid droplets was determined in the initial formulas and in the stomach and duodenal aspirates (just after collection) by using a particle size analyzer (Coulter LS 130, Coultronics, Margency, France). Mean sizes of emulsion lipid droplets were calculated by doing the mean of median size obtained after at least five measurements. In preliminary experiments, vegetable particles had been shown to affect the measurements, so they were discarded beforehand by centrifuging at 1,000 *g* for 2 min at 10°C.

Separation of fat and aqueous phases from the vegetable particles present in the GI content samples. To assess the transfer of carotenoids from the vegetable matrices to the fat and aqueous phases present in the GI contents, we separated these two phases from the vegetable matrices. For the stomach content samples, the protocol was as follows: 7 ml of stomach contents were placed in polyallomer tubes and centrifuged (2,000 *g*, 10 min, at 4°C). An aliquot (a few milligrams) of the floating oil phase, when present, was collected to measure carotenoid solubilized into the fat phase of the stomach. An aliquot of the infranatant was filtered through a sintered glass filter tube (100 mm high, 20 mm in diameter, 40- to 100-μm pore size) (Prolabo, Fontenay-sous-Bois, France) to discard large (>40 μm) particles of vegetable matrix. Liposomes and other aqueous soluble structures (phospholipid vesicles, proteins), which can potentially solubilize carotenoids in the aqueous phase of the stomach, were separated from small (<40 μm) fat globules and small vegetable particles (<40 μm) by ultracentrifuge (200,000 *g*, 335 min, 10°C, in a Kontron TST 41.14 swinging bucket rotor) followed by filtration of the infranatant through a 0.2-μm cellulose acetate filter (Schleicher & Schuell, Prolabo, Fontenay-sous-Bois, France).

For the duodenal samples, the protocol was as follows: 4 ml duodenal content were added with 7 ml cold (4°C) distilled water and placed in polyallomer tubes. The tubes were ultracentrifuged (same conditions as above) to float large lipid droplets and pellet vegetable particles. We noted that, in most of the duodenal samples, no floating oil layer was observed after the centrifugation. An aliquot of the infranatant was collected and filtered through a 0.2-μm cellulose acetate filter to discard small fat globules and vegetable particles and measure carotenoids in micelles. Control optical microscopy showed there were no oil droplets or vegetable particles in the aqueous phase obtained after this filtration.

Lipid analysis. Lipids from stomach and duodenal samples were extracted in chloroform/methanol [2:1 (vol/vol)] (13). The lower chloroform phases were evaporated to dryness under nitrogen. Total lipids were determined gravimetrically. The extent of intragastric and intraduodenal triacylglycerol lipolysis was assessed at 40 min after meal intake in the stomach and at 1 h after meal intake in the duodenum. For that purpose triacylglycerols were separated from other lipid classes (diacylglycerols, monoacylglycerols, free fatty acids, free cholesterol, and esterified cholesterol) by two-stage, one-dimensional thin-layer chromatography as described previously (1). They were quantified by densitometry by using a video-densitometry system and the Biolab software package (Visiosoft LND-CNRS patent, Marseille, France).

Chylomicron triacylglycerols were assayed by using an enzymatic colorimetric method with a commercial kit (Biotrol Diagnostic, Chennevières-lès-Louvres, France). The concentrations were measured spectrophotometrically at 490 nm by using an MR 700 microplate reader (Dsynatech Laboratories, Guernsey, UK).

Carotenoid analyses. A procedure to accurately extract carotenoids from the three different vegetable matrices and the stomach and duodenal samples was drawn up after preliminary experiments. Several combinations of solvents were tried, with the best combination being the one that gave the whitest vegetable matrix after extraction. The procedure, which was performed under yellow light, was as follows: 1-ml test meal or 2-ml stomach samples or 1-ml duodenal samples were added with 7 ml methanol containing 0.57% $MgCO_3$ (Sigma, Saint Louis, MO) and 0.2 μg/ml internal standard (echinenone, Roche Vitamines France, Neuilly-sur-Seine, France). After homogenization for 30 s with a vortex blender, 7 ml chloroform (containing 0.005% butylated hydroxytoluene as an antioxidant) were added. The sample was homogenized again for 30 s with the vortex blender. After 15 min rest, 7 ml distilled water were added. After being centrifuged (2,000 *g*, 10 min, room temperature), the lower phase containing most of the carotenoids was collected. Carotenoids remaining in the upper phase were extracted as follows: 5 ml tetrahydrofuran was added, the mixture was then vortexed for 30 s, and dichloromethane (5 ml) was added. It was then vortexed for 30 s, distilled water (3 ml) was added, and it was vortexed again for 30 s. After being centrifuged (2,000 *g*, 10 min, room temperature), the lower phase was collected and pooled with the previously collected phase. After evaporation to dryness under nitrogen, the dried extract was dissolved in either 200 μl acetonitrile/dichloromethane [50:50 (vol/vol)] for the stomach and test meal samples or 200 μl methanol/dichloromethane [65:35 (vol/vol)] for the duodenal samples. Chylomicron carotenoids were extracted by using a previously published method, i.e., twice with ethanol and hexane (29).

Carotenoids were quantified by reverse-phase HPLC on a Waters system (Waters, Saint-Quentin-en-Yvelines, France). This system was composed of a Waters 660 pump, a Waters 717 plus cooled auto-sampler, and a Waters 996 ultraviolet (UV)-visible diode-array detector. Carotenoids were separated by using two columns fitted in series (41): a 150 × 4.6-nm RP C_{18}, 3-μm Nucleosyl (Interchim, Montluçon, France) coupled with a 250 × 4.6-nm RP C_{18}, 5-μm Vydac TP54 (Hesperia, CA). The mobile phase was an isocratic acetonitrile-dichloromethane-methanol (containing 50 mM ammonium acetate)-water mixture (70:10:15:5, by vol). Carotenoids were detected at 450 nm and identified by retention time and spectral analysis (from 300 to 550 nm) compared with pure (>95%) standards of the following carotenoids: lutein, echinenone, all-*trans* lycopene, α-carotene, all-*trans* β-carotene, and 13-*cis* β-carotene (Roche Vitamines). Because we had no pure standard of *cis* isomers of lycopene, the main *cis* isomers of lycopene (in terms of peak area), which were identified by their characteristic UV/Vis spectrum (with the small hypochromic shift in λ_{max} and the presence of a "*cis* peak" at ~142 nm below the longest wavelength absorption maximum), were quantified by using the calibration curve of all-*trans* lycopene and called lycopene *cis*-isomers. Quantification was conducted by using Waters Millenium 32 software (version 3.05.01). The detection threshold was measured at ~2 ng carotenoid/HPLC injection. With the use of an internal standard, we calculated an overall recovery yield of 75–

100%. All the solvents used for carotenoid analyses were HPLC grade from Carlo Erba (Chaussée de Vexin, France).

Calculations and Statistical Analysis

The proportions of carotenoids remaining in the vegetable matrices in the stomach and duodenal contents were calculated by subtracting the carotenoids recovered in the lipid phase and in the aqueous phase from the total carotenoids measured in the samples. The extent of intragastric and intraduodenal triacylglycerol lipolysis was calculated as the percentage of triacylglycerols disappearing from the total acylglycerols present (triacylglycerols + diacylglycerols + monoacylglycerols). The area under the curves (AUCs) of the postprandial chylomicron responses (delta from fasting values) were calculated by the trapezoidal rule.

Subject characteritics and results are expressed as means ± SE. Data were tested for normality (Kolmogorov-Smirnov) before using parametric tests. Changes in measured parameters were analyzed by ANOVA. Two-factor ANOVA, with time and meal as factors, was used to study variations in stomach and duodenal pH during digestion. Variations in *cis* β-carotene and lycopene ratio during digestion were assessed by using two-factor ANOVA, with time and organ as factors. Proportions of carotenoids in micellar phase were compared by using two-factor ANOVA, with time and carotenoid type as factors. Variations in postprandial chylomicron carotenoid concentrations were assessed by ANOVA with repeated measures, with time as a factor. When significant ($P < 0.05$) differences were detected, means were compared between each other by using the post hoc Tukey/Kramer's test. Pearson correlation coefficients were obtained from linear regression analyses. Statistics were performed using StatView software version 5.0 (SAS Institute, Cary, NC).

RESULTS

Stomach and Duodenum pH During Digestion

Figure 1 shows variations in pH in stomach and duodenum contents after the intake of tomato, carrot, and spinach meals. As shown in Fig. 1*A*, the stomach pH, which was ~1.8 in the fasting state, sharply increased to 5.4–6.2 after meal intake, then continuously decreased to reach 1.8–2.9 after 3 h digestion. A two-factor ANOVA showed there was no meal effect but a time effect on postprandial stomach pH. The duodenal pH (Fig. 1*B*), which was ~5 in the fasting state, increased to ~6.1–6.6 after meal intake, and remained constant during digestion. Two-factor ANOVA showed a meal effect and no time effect on postprandial duodenal pH. More precisely, the mean postprandial duodenal pH was significantly lower after the tomato meal (5.8 ± 0.1) than after the other two meals (6.2 ± 0.1 and 6.4 ± 0.1 for the spinach and the carrot meal, respectively).

Size of Emulsion Lipid Droplets in Test Meals and in Stomach and Duodenal Contents During Digestion

In the test meals and in the stomach, lipid droplets exhibited monophasic distributions with bell-shaped curves (data not shown in the figures). In the duodenum, small droplets appeared (size 0.1–1 μm) resulting in biphasic distributions. The mean diameter of lipid droplets found in the carrot, tomato, and spinach meals was significantly different: 10.4 ± 0.4, 2.7 ± 0.2, and 8.1 ± 0.8 μm, respectively. The mean diameter of lipid droplets found in the stomach after the carrot meal (mean of 3 measurements made at each collection time) was significantly higher than that measured after the tomato and the spinach meals: 15.1 ± 0.7 vs. 6.1 ± 0.9 and 7.9 ± 0.5 μm, respectively. The mean diameters of lipid droplets found in the duodenum after the carrot, the tomato, and the spinach test meals were 11.6 ± 1.6, 5.2 ± 0.7, and 4.8 ± 0.6 μm, respectively.

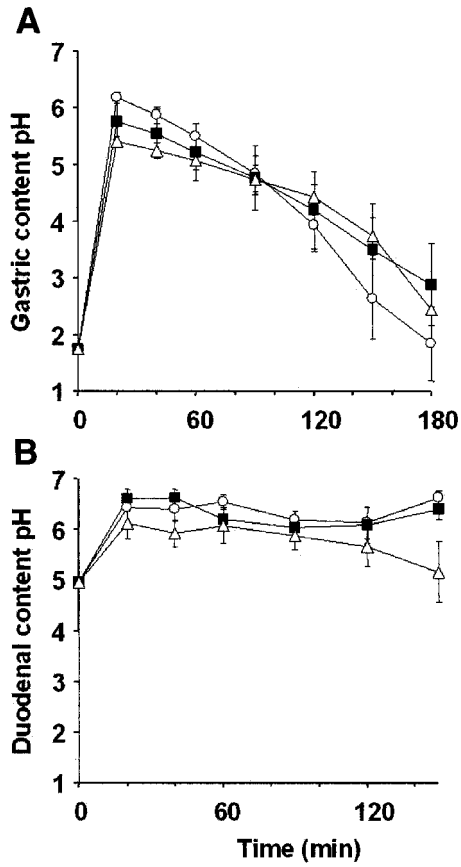

Fig. 1. Variations in pH in the upper gastrointestinal (GI) tract content during digestion. *A*: stomach content. *B*: duodenal content. ○, Carrot meal; ■, spinach meal; △, tomato meal. Values represent means ± SE of 10 separate measurements. Two-factor ANOVA, with time and meal as factors, showed no effect of meal on stomach pH but a significant ($P < 0.001$) effect of meal on duodenal pH. It also showed a significant effect of time ($P < 0.001$) on stomach pH but not on duodenal pH.

Triacylglycerol Lipolysis

The relative amount of triacylglycerols present in the gastric aspirates at 40 min decreased by 12.6 ± 1.8, 14.3 ± 2.5, and 21.8 ± 3.9% for the carrot, tomato, and spinach meal, respectively. In the 1-h duodenal contents, triacylglycerol disappearance was more marked: 48.9 ± 4.7, 56.7 ± 4.2, and 47.0 ± 9.2% for the carrot, tomato, and spinach meal, respectively.

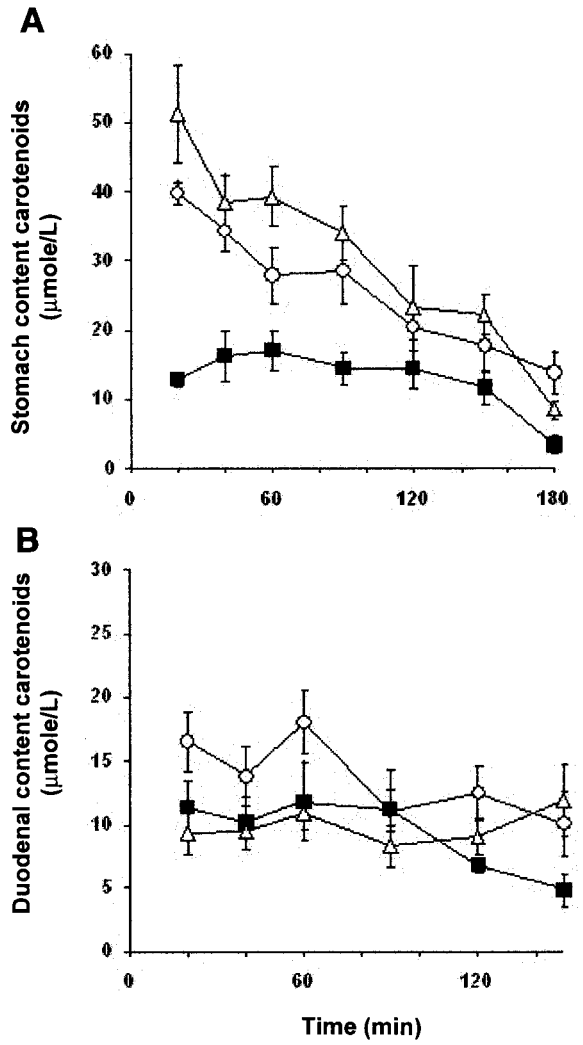

Fig. 2. Stomach and duodenal carotenoid concentrations during digestion. *A*: stomach content. *B*: duodenal content. ■, Lutein (after the spinach meal); ○, β-carotene (after the carrot meal); △, lycopene (after the tomato meal). Values are means ± SE.

Cartenoids in Fasting Stomach and Duodenal Contents

After fasting, no carotenoids were detectable in the samples of stomach contents. Thus assuming a limited detection threshold of 2 ng carotenoid per sample and given that the measurements were made on 2-ml stomach samples, we can assert that the concentration of carotenoids in the fasting stomach was lower than 2 nM. Conversely, carotenoids were detected in the fasting duodenum samples. Specifically, there were detectable amounts of lutein and all-*trans* β-carotene (155 ± 51 and 30 ± 9 nM, respectively) and no detectable amounts, i.e., <4 nM (the measurements were carried out with 1 ml duodenum content), of α-carotene, lycopene *cis* isomers, and β-carotene *cis* isomers.

Concentration of Carotenoids in Stomach and Duodenum During Digestion

Figure 2 shows that the concentration of carotenoids in the stomach gradually decreased during digestion. Conversely, it ranged between 5 and 18 μM in the duodenum and did not significantly vary during digestion. Stomach lutein concentration was significantly lower than β-carotene and lycopene stomach concentrations. Conversely, in the duodenum, there was no significant difference in the concentration of each carotenoid.

Proportion of cis *Isomers of β-Carotene and Lycopene in Stomach and Duodenum During Digestion*

Although the proportion of *cis* carotenoids in the stomach and in the duodenum did not vary significantly during digestion, it was significantly higher in the duodenum than in the stomach (Fig. 3). Specifically, the mean postprandial proportions of 13-*cis* β-carotene were 12.2 ± 0.6 and 8.0 ± 1.5% in the duodenum and the stomach, respectively, and the mean postprandial proportions of *cis* lycopene were 13.6 ± 0.9 and 11.1 ± 0.9%.

Distribution of Carotenoids in Different Phases Present in Stomach During Digestion

The distribution of all-*trans* β-carotene, lycopene, and lutein between the vegetable matrix, fat phase,

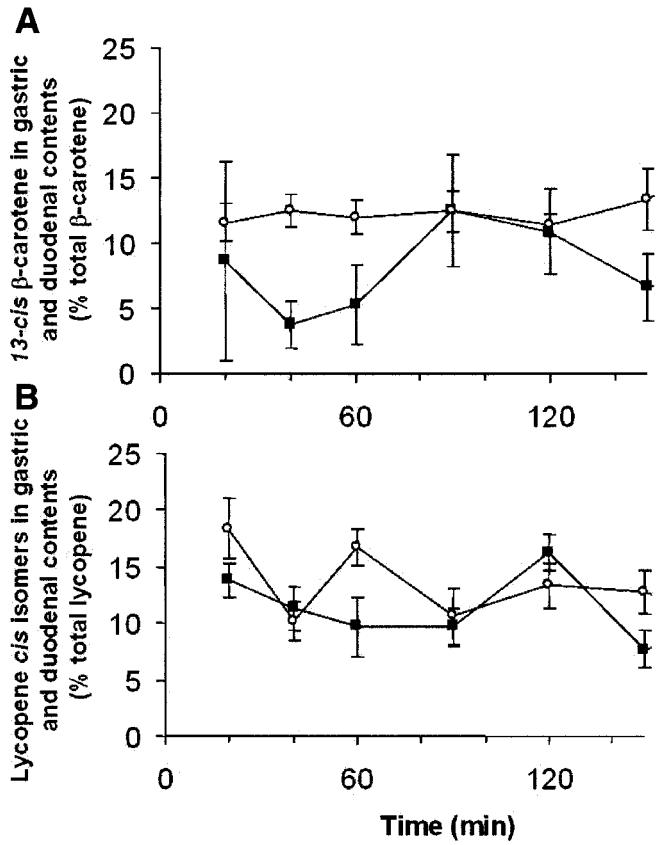

Fig. 3. Proportion of carotenoid *cis* isomers in the stomach (■) and in the duodenum (○) during digestion. *A*: 13-*cis* β-carotene from the carrot meal. *B*: lycopene *cis* isomers from the tomato meal. Values represent means ± SE. ANOVA showed an effect of organ ($P < 0.05$) on the proportion of *cis* carotenoids in the GI contents. The post hoc Tukey-Kramer test showed that the 20- to 150-min mean proportion of carotenoid *cis* isomers was significantly higher in the duodenum than in the stomach.

and aqueous phase present in the stomach during digestion is shown in Fig. 4. The first striking observation was that only trace amounts (0–1.2%) of carotenoids were recovered in the aqueous phase of the stomach, whatever the digestion time. The second observation was that the proportion of all-*trans* β-carotene in the carrot matrix continuously decreased during digestion, whereas it increased in the fat phase. We noted that the results obtained for α-carotene provided in the carrot meal were similar to those of all-*trans* β-carotene from the same meal (data not shown in the figure). Concerning all-*trans* lycopene, most (86–95%) of it remained in the tomato matrix during digestion, and only ~6% was recovered in the fat phase. Finally, all-*trans* lutein exhibited an intermediate figure, with 70–80% of this carotenoid remaining in the spinach matrix and 20–30% rapidly recovered in the fat phase.

Proportion of Carotenoids in Aqueous (Micellar) Phase Present in Duodenum

Only a very small proportion (between 1.8 and 6.9%) of all-*trans* carotenoids was recovered in the aqueous phase of the duodenum (Fig. 5). There was an effect of carotenoid species on the proportion of carotenoid found in the aqueous phase. More precisely, the 20- to 150-min mean proportions of lutein and all-*trans* β-carotene in the aqueous phase (5.6 ± 0.4 and 4.8 ± 0.3%, respectively) were significantly higher than that of all-*trans* lycopene (2.0 ± 0.2%). The proportion of α-carotene in the aqueous phase of the duodenum after the intake of the carrot meal was 4.7 ± 0.6% (data not shown in the figure).

Figure 6 shows that the 20- to 150-min mean proportions of 13-*cis* β-carotene recovered in the aqueous phase of the duodenum were significantly ($P < 0.001$) higher than that of all-*trans* β-carotene: 14.8 ± 1.6 vs. 4.7 ± 0.3%. The results of lycopene *cis* isomers are not presented here owing to the very high variability of the data obtained.

Postprandial Chylomicron Lipid and Carotenoid Responses to Test Meals

The postprandial chylomicron triacylglycerol responses, as estimated by 0–8 h AUC, were not significantly different among the three test meals: 0.58 ± 0.10, 0.60 ± 0.09, and 0.54 ± 0.10 mM/h for the tomato, carrot, and spinach meal, respectively (data not shown in the figures). For carotenoids, the first observation was that, although there was detectable all-*trans* lycopene in the chylomicron fraction, there was no significant variation in the postprandial all-*trans* lycopene response to the tomato test meal (as assessed by ANOVA with repeated measures, with time as a factor; Fig. 7). In contrast, there was an effect of time on the all-*trans* β-carotene concentrations in the chylomi-

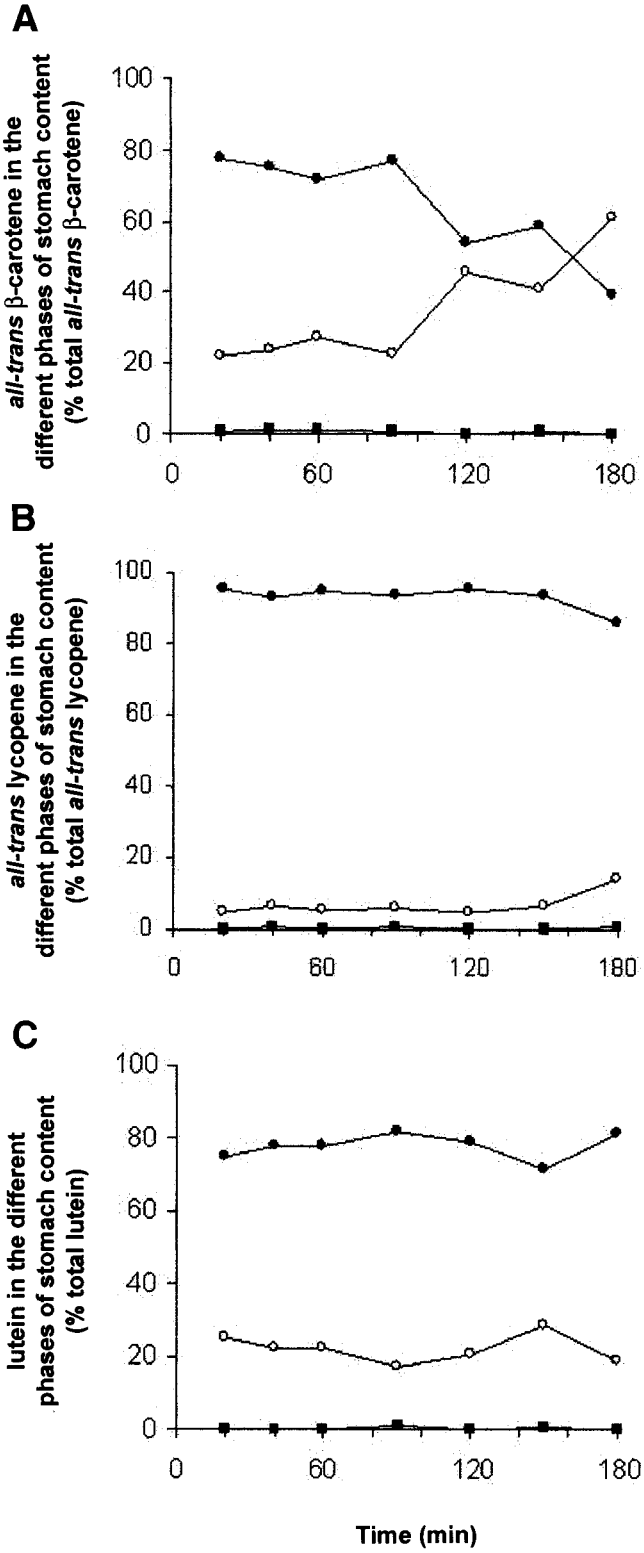

Fig. 4. Proportion (%) of all-*trans* carotenoids recovered in the vegetable matrix (●), in the fat phase (○), and in the aqueous phase (■) of stomach contents during digestion. *A*: all-*trans* β-carotene (measured after ingestion of the carrot meal). *B*: all-*trans* lycopene (after the tomato meal). *C*: all-*trans* lutein (after the spinach meal). Carotenoids were measured in the whole samples, in the fat, and in the aqueous phase of the samples. The proportion of carotenoids in the vegetable matrix was calculated by subtracting the amount of carotenoids recovered in the fat and aqueous phases from the whole amount measured in the samples. Percentages were calculated from means of 10 measurements.

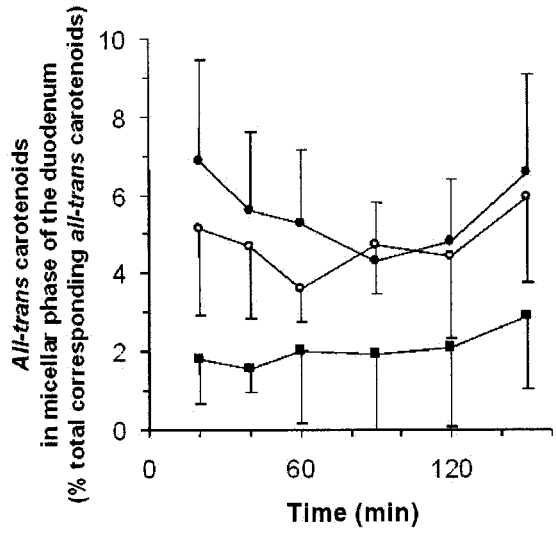

Fig. 5. Proportion (%) of all-*trans* carotenoids in the aqueous (micellar) phase of duodenal content during digestion. ●, Lutein (after the spinach meal); ○ all-*trans* β-carotene (after the carrot meal); ■, all-*trans* lycopene (after the tomato meal). ANOVA showed an effect of carotenoid type ($P < 0.0001$) on the 20- to 150-min mean proportion of carotenoids in this phase of the duodenum. The post hoc Tukey-Kramer test showed that the 20- to 150-min mean proportion of all-*trans* lycopene was significantly lower than that of the 2 other carotenoids.

crons after the carrot meal and on the lutein concentrations in the chylomicrons after the spinach meal (Fig. 7). In fact, there was no significant difference between the all-*trans* β-carotene response (0- to 8-h AUC) obtained after ingestion of the carrot meal (24.2 ± 4.3 nM·h) and the lutein response obtained after ingestion of the spinach meal (24.0 ± 4.3 nM·h). The second observation was that 13-*cis* β-carotene was detected in most (>63%) of the chylomicron samples measured after ingestion of the carrot meal, resulting in a significant variation in the concentration of the

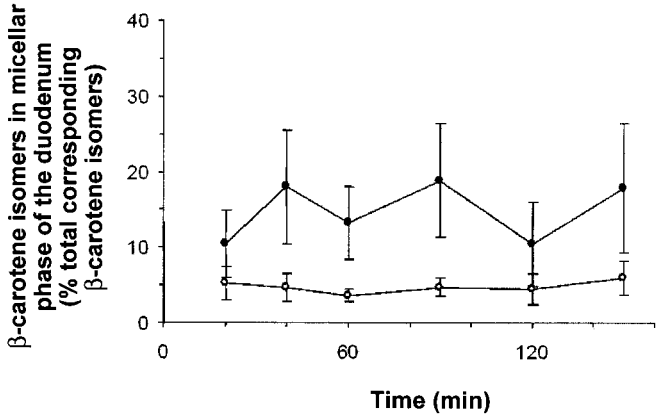

Fig. 6. Proportion (%) of 13-*cis* (●) and all-*trans* (○) β-carotene in the aqueous (micellar) phase of the duodenum. Values represent means ± SE of 10 measurements. ANOVA showed no time effect but an effect of β-carotene isomer ($P < 0.0001$) on the 20- to 150-min mean proportion of β-carotene in this phase of the duodenum. The post hoc Tukey-Kramer test showed that the 20- to 150-min mean proportion of 13-*cis* β-carotene was significantly higher than that of the all-*trans* isomer.

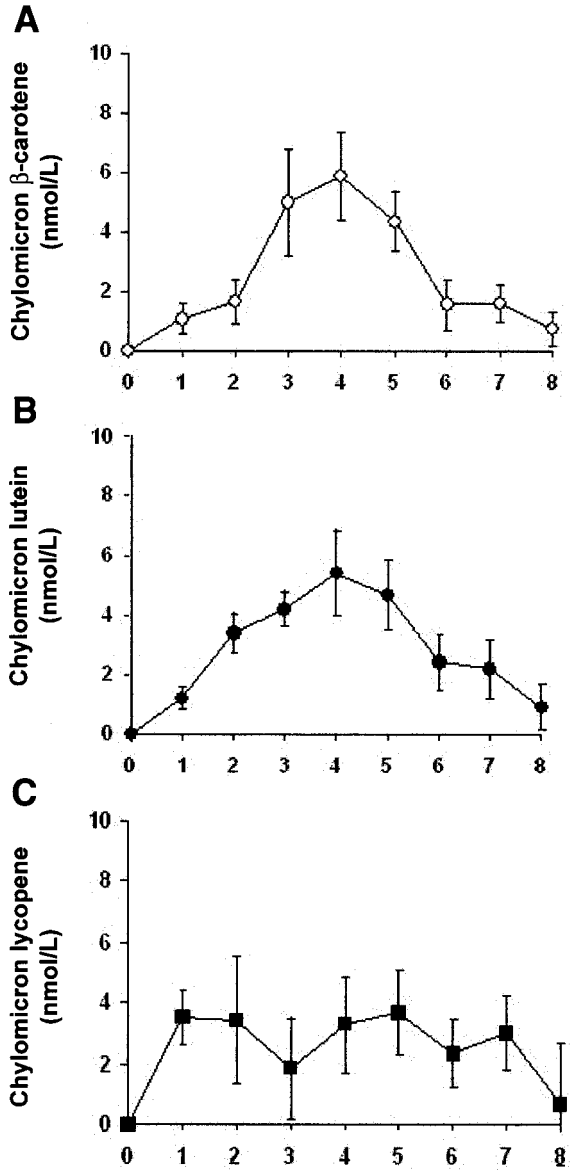

Fig. 7. Postprandial chylomicron carotenoid responses to the test meal. ○, All-*trans* β-carotene (after the carrot meal); ●, lutein (after the spinach meal); ■, all-*trans* lycopene (after the tomato meal). Values (changes from fasting values) represent means ± SE of 10 measurements. ANOVA showed a time effect on chylomicron β-carotene and lutein concentrations but no time effect on chylomicron lycopene concentrations.

13-*cis* β-carotene in the postprandial period. Thus the calculated 13-*cis* β-carotene response was 2.8 ± 0.9 nM·h. In contrast, although trace amounts of lycopene *cis* isomers were detected in some chylomicron samples (~20% of the samples), most samples did not show detectable lycopene *cis* isomers, so there was no significant variation in the concentration of lycopene *cis* isomers in the chylomicrons after ingestion of the tomato meal.

DISCUSSION

The aim of this study was to obtain basic data on the fate of carotenoids in the human upper GI tract. Spe-

cifically, we wanted to determine *1*) whether there is a *trans*-to-*cis* isomerization of β-carotene and lycopene in the acidic environment of the stomach, *2*) whether the stomach plays a role in the bioavailability of carotenoids, *3*) how efficient the transfer of carotenoids from their vegetable matrix to micelles is, and *4*) whether *cis* and *trans* isomers of carotenoids have different metabolisms in the gut lumen. To answer these questions, we followed the processing of carotenoids in the human upper GI tract by collecting chyme samples during the digestion of liquid test meals. This approach has already been used to study the metabolism of fat soluble vitamins in the human upper GI tract (5).

The first observation concerns the concentration of carotenoids in the stomach and duodenum during digestion. Curves exhibited by β-carotene and lycopene in the stomach were expected, with a gradual decrease in carotenoid concentration explained by the gastric emptying of the meals. The curve of lutein was significantly lower. It is difficult to explain such a difference, because the same amount of the main carotenoids was provided in each meal. The most likely hypothesis is that, although large samples of stomach content were collected to have a representative sample, the distribution of the vegetable matrix, i.e., chopped spinach leaves, was inhomogeneous in the stomach leading to an underestimation of lutein concentration. The relatively constant concentration of carotenoids in the duodenum can be explained by an equilibrium between the entry of carotenoids coming from the stomach and the exit of carotenoids either absorbed or transfered in the lower parts of the intestine.

Given that isomerization of *trans* carotenoids to *cis* carotenoids is promoted by contact with acids (36), our first goal was to assess whether there was any isomerization of carotenoids in the acidic environment of the stomach. The lack of any significant increase in the *cis-trans* β-carotene and *cis-trans* lycopene ratios in the stomach during digestion led to the conclusion that there was no significant *cis-trans* isomerization of these carotenoids in the human stomach. This result seems at variance with a recent result obtained in vitro (34), which showed an effect of acidic pH on lycopene isomerization. However, in that study, the isomerization was observed at the very low pH of 1.6, a value found only in the fasting stomach and not during the digestion of the complex lycopene-rich meal used in this study (see Fig. 1).

The fact that the *cis-trans* β-carotene and lycopene ratios were significantly higher in the duodenum than in the stomach was noteworthy. A first hypothesis is that there was a lower bioaccessibility (defined as the ease with which the carotenoids are solubilized within the mixed micelles from the vegetable matrix) of the *cis* isomers compared with the *trans* isomers, resulting in a lower absorption efficiency for the *cis* isomers. However, this is unlikely because there was a higher proportion of 13-*cis* β-carotene than of all-*trans* β-carotene in the aqueous phase of the duodenum (Fig. 6), suggesting a better bioaccessibility of the *cis* isomers, and it has been shown that, when artificially incorporated in micelles, both isomers are absorbed with the same efficiency. A second hypothesis is that *cis* isomers were secreted in the bile, resulting in an increased *cis-trans* ratio in the duodenum. However, although we detected all-*trans* β-carotene and lutein in the fasting duodenal samples, in agreement with previous results (24), we did not detect significant amounts of carotenoid *cis* isomers in the fasting samples of the duodenum. A third hypothesis is that there was a *cis*-to-*trans* isomerization of carotenoids in the duodenum, but we have no evidence of this.

Because the carotenoids were initially present only in the vegetable matrices, their presence in the fat phase of the stomach content must have resulted from their transfer from the vegetable matrices to this phase. Although such a transfer has been described in vitro (35), this is the first time that it has been measured in humans. The data collected show that the different carotenoids were transferred with different efficiencies. This may be explained in several ways. The in vitro results (35) suggest that there was an effect of pH on the transfer efficiency. However, this hypothesis can be rejected, because there was no significant meal effect on stomach pH. A second hypothesis is that there was a matrix effect, i.e., that the different vegetable matrices (carrot, spinach, and tomato) have different abilities to release carotenoids (due to different fiber composition or different intracellular locations of carotenoids). This second hypothesis can also be rejected because the kinetics of transfer of β-carotene from spinach (data not shown in the figures) mimicked that of β-carotene from carrots and was very different from that of lutein from spinach. A third hypothesis is that there was an effect of triacylglycerol lipolysis on the transfer. However, although there was a significant matrix effect on gastric triacylglycerol lipolysis, there was no relationship between the proportion of lipids remaining as triacylglycerols, which reflects triacylglycerol lipolysis efficiency, and transfer efficiency. A fourth hypothesis is that the transfer of carotenoids depends on their relative solubility in the fat phase. This hypothesis seems plausible because there was a strongly positive relationship ($r = 0.99$, $P = 0.01$) between carotenoid solubility in tri-C18 triacylglycerols (3) and the proportion of carotenoids in the fat phase at 180 min digestion. The last hypothesis is that the size of the lipid droplets, and thus the area for exchange, affected the transfer efficiency. The positive relationship ($r = 0.98$, $P = 0.08$) between the mean diameter of emulsions recovered in the stomach and the transfer efficiency at 180 min suggested that some parameter related to the quality of the interface might also be involved in the transfer. Whatever the mechanism involved, these data suggest that the stomach plays an important role in the bioaccessibility of carotenoids by initiating their release from their vegetable matrices.

Because it is assumed that carotenoids are absorbed only when they are solubilized in micelles, the very low percentage of carotenoids recovered in the aqueous (micellar) phase of the duodenum suggests that only a

very low proportion of carotenoids, supplied in their natural vegetable matrix, is available for absorption. This result is in remarkable agreement with a recent study performed in CaCo-2 (10). Indeed it was shown that the extent of absorption of carotenoids was comprised of between 11% for all-*trans* β-carotene and 2.5% for all-*trans* lycopene. It is also in agreement with a study that estimated absorption efficiency from chylomicron response (32). With this method, it was estimated that 1.4 mg of 40 mg β-carotene (3.5%) and 1 mg of 40 mg lycopene (2.5%) were absorbed in humans, values close to the solubility values measured here, i.e., 4.8 and 2.0%. However, note that these solubility values would have been probably different if purified carotenoids were used. Indeed, it is well known that carotenoids compete for absorption (42, 44) and the vegetables ingested contained several carotenoids (see MATERIALS AND METHODS). However, the aim of this study was to provide basic data on the digestion of vegetable-borne carotenoids, which are mostly recovered with other carotenoids species in vegetables.

The fact that the percentage of all-*trans* lycopene recovered in the micellar phase of the duodenum was significantly lower than that of all-*trans* β-carotene and lutein was noteworthy. It is in remarkable agreement with a recent result that showed that the extent of absorption of β-carotene, α-carotene, and lutein by CaCo-2 cells was higher than that of lycopene (10). It suggests that the availability for absorption of lycopene is less efficient than that of the other carotenoids. The question arises whether this difference depends on a vegetable matrix effect or on some other effect. Although it was not possible to accurately measure the proportion of β-carotene in micelles after the tomato meal (due to the very low amount of β-carotene provided by this meal, i.e., ~1 mg), the fact that the proportion of β-carotene from spinach was similar to that of β-carotene from carrot (data not shown in the figures) suggests that the efficiency of transfer to micelles does not depend on vegetable matrix characteristics. Because carotenoids are assumed to be entrapped in the fat phase of dietary emulsions and because it has been suggested that triacylglycerol lipolysis enhances the release of the carotenes (3, 43), we suggested that the efficiency of triacylglycerol lipolysis affects the carotenoid transfer to micelles. This hypothesis seemed valid, because there was a strong negative relationship ($r = -0.998$, $P = 0.036$) between the concentration of triacylglycerols in the duodenum, which reflects lipolysis, and the percentage of carotenoids found in the micellar phase at 1 h of digestion. The lowest solubility of lycopene in the micellar phase may result from the lower pH observed in the duodenum during the digestion of the tomato meal (Fig. 1). It has recently been shown that the efficiency of carotenoid transfer from emulsion lipid droplets to micelles diminishes as the pH decreases (43). It may also result from its higher hydrophobicity. It is remarkable that the least hydrophobic carotenoid (7), lutein, had the highest solubility in micelles, whereas the most hydrophobic carotenoid, lycopene, had the lowest solubility. This hypothesis is in agreement with previous results (14, 45) that found a higher relative bioavailability of lutein compared with β-carotene.

The higher proportion of 13-*cis* β-carotene in the micellar phase may be due to either a higher micelle transfer efficiency, a lower absorption efficiency of micellar 13-*cis* β-carotene, or both. This hypothesis is supported by the fact that the absorption efficiency of 13-*cis* β-carotene is significantly lower than that of the all-*trans* isomer (10) and by the fact (27) that the *cis* isomer is more efficiently transferred to micelles than the all-*trans* isomer. The reason for this is unknown but could be due to either a different partitioning of the two isomers between the core and the surface of emulsion lipid droplets (3) or different solubilities of the two isomers in micelles. Finally, the fact that the 13-*cis*-to-all-*trans* β-carotene ratio measured in the micellar phase (13.3) was very close to the 13-*cis*-to-all-*trans* β-carotene ratio measured in the chylomicrons (11.5) suggests that 13-*cis* β-carotene does not undergo the *cis*-to-*trans* isomerization previously demonstrated for 9-*cis* β-carotene (48) in the enterocyte.

Before discussion on the postprandial chylomicron carotenoid responses, it should be stated that these responses could not be compared in intensity with those obtained in other studies with similar doses of lipids and carotenoids, because an important proportion of lipids and carotenoids was taken in the gut during digestion for the different analysis. The fact that chylomicron all-*trans* lycopene did not significantly vary in the postprandial period, whereas all-*trans* β-carotene and lutein did, is in remarkable agreement with the data obtained in the duodenum, with a lower solubility of lycopene in micelles compared with the two other carotenoids. The fact that *cis* isomers of lycopene were hardly detected in chylomicrons can be explained by a low absorption efficiency, an isomerization to the *trans* isomer in the enterocyte, or a specific transport via the portal pathway. The first hypothesis can reasonably be rejected, because it has been suggested that *cis* isomers of lycopene artificially incorporated in micelles are more bioavailable than all-*trans* isomers of lycopene (2). The fact that *cis* isomers of lycopene are detected in human plasma and tissues (18, 38, 40), accounting for >50% total plasma lycopene (38), plus the fact that our data suggest that they are poorly transported by the chylomicrons and thus poorly absorbed strongly suggests that a *trans*-to-*cis* isomerization of this carotenoid, possibly due to antioxidant reactions of lycopene (30), occurs in the body at a postenterocyte level.

In conclusion, this study provides some new data on the fate of carotenoids in the human upper GI tract. The main findings are that *1*) there is no significant *cis-trans* isomerization of β-carotene and lycopene in the human stomach, *2*) the stomach plays a significant role in the bioavailability of carotenoids by initiating their transfer from the vegetable matrix to the fat phase of the meal, *3*) the proportion of carotenoids recovered in the micellar phase of the duodenum is very low (<7%), which probably explains the poor bio-

availability of these phytomicroconstituents, *4*) 13-*cis* β-carotene is more fully solubilized in micelles than the all-*trans* isomer, explaining its higher bioavailability, and *5*) *cis* isomers of lycopene are sparingly secreted in the chylomicrons, suggesting a postenterocyte origin for these isomers in the body.

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