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Bioaccessibility of Carotenoids and Vitamin E from Their Main Dietary Sources

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Vitamin E and carotenoids are fat-soluble microconstituents that may exert beneficial effects in humans, including protection against cancer, cardiovascular diseases, and age-related eye diseases. Their bioavailability is influenced by various factors including food matrix, formulation, and food processing. Since human studies are labor-intensive, time-consuming, and expensive, the *in vitro* model used in this study is increasingly being used to estimate bioaccessibility of these microconstituents. However, the ability of this model to predict bioavailability in a healthy human population has not yet been verified. The first aim of this study was to validate this model by comparing model-derived bioaccessibility data with (i) human-derived bioaccessibility data and (ii) published mean bioavailability data reported in studies involving healthy humans. The second aim was to use it to measure α- and γ-tocopherol, β-carotene, lycopene, and lutein bioaccessibility from their main dietary sources. Bioaccessibility as assessed with the *in vitro* model was well correlated with human-derived bioaccessibility values ($r = 0.90$, $p < 0.05$), as well as relative mean bioavailability values reported in healthy human groups ($r = 0.98$, $p < 0.001$). The bioaccessibility of carotenoids and vitamin E from the main dietary sources was highly variable, ranging from less than 0.1% (β-carotene from raw tomato) to almost 100% (α-tocopherol from white bread). Bioaccessibility was dependent on (i) microconstituent species (lutein > β-carotene and α-carotene > lycopene and α-tocopherol generally > γ-tocopherol), (ii) food matrix, and (iii) food processing.

KEYWORDS: Micelles; absorption; bioavailability; food processing; bioaccessibility; human

INTRODUCTION

The absorption efficiency of highly lipophilic food microconstituents (HLFM), i.e., microconstituents with octanol−water partition coefficients log $p_c > 8$ (vitamins A, E, D, and K, carotenoids and phytosterols (*1*); is highly variable and dependent on a range of factors, one of the most important being food matrix characteristics (*1*). The results of numerous epidemiological studies suggest that these molecules exert beneficial health effects. However, health benefits sometimes correlate less with food intake than with plasma concentrations of these molecules. This is in part due to errors in estimate of intake because of huge variations in the HLFM content of foods and because dietary tables do not take into consideration the absorption efficiency of these molecules which are present in various food matrices (*2, 3*).

Several models are available to estimate HLFM absorption efficiency. Some estimate bioaccessibility, other uptake by intestinal cells, other availability, and other bioavailability. Bioaccessibility, i.e., the fraction of HLFM transferred from the food matrix to micelles, is estimated by an *in vitro* digestion model (*4*). Uptake by intestinal cells is estimated using human intestinal cell lines (*5*), animal brush border membrane vesicles (*6*), or animal intestinal everted sacs (*7*). Availability, i.e., the fraction absorbed and recovered in the lymph, is estimated using either an artificial gastrointestinal tract model (*8*) or an *in situ* rat intestinal perfusion and lymph collection model (*9*). Finally, bioavailability, i.e., the fraction of newly ingested HLFM recovered in plasma, is estimated using animal models or in human studies by measuring long-term plasma responses (*10*), by postprandial chylomicron responses (*11*) or by measuring the appearance of stable isotopes of HLFM in the plasma (*12*−*17*). Each model presents advantages and limitations and there is no gold standard.

The "*in vitro* digestion model" (*4*) estimates bioaccessibility in simulated digestion of meals containing HLFM. Although this model is becoming increasing popular (*4, 18*−*22*), its ability to estimate the bioavailability of HLFM in healthy humans has not yet been verified. The aims of this study were therefore (1) to optimize the model in terms of human physiology, (2) to

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Table 1. Meal Composition[a]

component	amount (g)	preparation	provider
pureed potatoes	6.7	boiled	food supermarket
minced beef	1.2	fried	food supermarket
olive oil	0.2	unprocessed	food supermarket
HLFM-rich food/ supplement	0.05-4	variable	variable

[a] The test meal without the HLFM-rich source contained 53.6% energy as carbohydrates, 28.4% as fat, and 18.0% as proteins. When, for example, 2 g tomato puree was added as a lycopene-rich source, the meal contained 54.7% energy as carbohydrates, 27.6% as fat, and 17.6% as proteins. These proportions are close to US-DRI, i.e., 45–65% carbohydrates, 20–35% fat, and 10–35% proteins.

check whether the model-derived bioaccessibility values were correlated to mean bioavailability values observed in published humans studies, and (3) to use the model to measure the bioaccessibility of carotenoids and vitamin E from their main dietary sources.

MATERIALS AND METHODS

Supplies. Foods were purchased from a local supermarket. Pepsin, porcine pancreatin, porcine bile extract, pyrogallol, and vitamin E (DL-α-tocopherol, D-γ-tocopherol, and DL-α-tocopherol acetate) were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Pure carotenoids (>91.7%) and carotenoid rich supplements (oil in water emulsions containing 30% β-carotene or 20% lutein) were kindly provided by DSM LTD (Basel, Switzerland).

Preparation of the Meals. Meal composition is given in **Table 1**. Potatoes were boiled in tap water, peeled, and hand-pureed. Meat was fried medium in a frying-pan without added fat. Potato puree and fried meat were divided into aliquots and frozen at −20 °C. Sources of HLFM were the main dietary sources of carotenoids and vitamin E (**Table 2**).

Simulated Digestion of the Meals. Some features of the initial model published by Garrett et al. (*4*, *18*) were modified to take into account recent data on lipid digestion and carotenoid processing in the human gastrointestinal tract (*23*). These features are listed below:

- Meal composition was modified in order to align macronutrient proportions to current RDAs (see **Table 1**). Indeed, since macronutrients affect HLFM bioavailability (*1*), it is important to use a meal that mimics the proportions of each macronutrient found in regular meals.
- β-Hydroxy toluene (BHT), which was used in the initial model as a preservative to protect antioxidant micronutrients from oxidation, was replaced by pyrogallol (*24*) because, in contrast with BHT, pyrogallol is readily soluble in water, making it more efficient in protecting HLFM solubilized in water-soluble vehicles (cells of the vegetable matrix and micelles).
- The pH of the gastric medium was adjusted to 4 instead of 2, because pH 2 is only found in the fasting state and the mean pH measured in the human stomach after ingestion of vegetable-rich meals ranges between 5.8 just after meal intake to 3 at 3 h later (*23*).
- The pH of the duodenal medium was set at 6.0 instead of 7.5 to match the pH measured in the human duodenum during digestion (*23*). Note that pH is a key factor governing the transfer of carotenoids from emulsion lipid droplets to micelles, which is a fundamental step in HLFM bioavailability (*25*).
- Duration of incubation in duodenal conditions was set at 30 min instead of 2 h to approach the digestive transit time of a food particle in the human duodenum (*26*).
- Bile salt concentrations in the duodenal conditions were increased because lycopene was barely soluble in micellar phase in the original study (*4*), and preliminary experiments had shown that an increase in bile salt concentrations led to accurate detection of lycopene in the micellar fraction.

Meal components (including HLFM) were mixed with 32 mL of NaCl 0.9% containing 12.6 mg/mL pyrogallol. The mixture was homogenized for 10 min at 37 °C in a shaking water bath. pH was adjusted to 4 ± 0.02 with about 500 μL of 1 M HCl, and then 2 mL of porcine pepsin (40 mg/mL in 0.1 M HCL) was added. The homogenate was incubated at 37 °C in a shaking water bath for 30 min. The pH of the partially digested mixture was raised to 6 ± 0.02 by adding around 800 μL of 0.9 M sodium bicarbonate pH 6.0. Then, a mixture of porcine bile extract and pancreatin (9 mL containing 2 mg/mL pancreatin and 12 mg/mL bile extract in 100 mmol/L trisodium citrate, pH 6.0) and 4 mL of porcine bile extract at 0.1 g/mL were added. Samples were incubated in a shaking water bath at 37 °C for 30 min to complete the digestion process.

Isolation of the Micellar Fraction from Digesta. Micelles were separated from oil droplets and food particles by ultracentrifugation (20000 rpm for 18 h at 10 °C in a Kontron TST 41−14 SW rotor). The aqueous fraction was collected from the centrifuge tube using a needle fitted to a 10 mL syringe. The aqueous fraction was passed through a 0.22 μm filter (Millipore). Aliquots were stored at −80 °C under a blanket of nitrogen until analysis.

Analysis of Carotenoids and Vitamin E. Carotenoids and vitamin E were extracted from the digesta and micellar fraction as previously described (*23*). The procedure was as follows: 1 mL of sample was added to 7 mL of methanol containing 0.57% $MgCO_3$ (Sigma, St. Louis, MO) and 0.2 μg/mL internal standard (echinenone). After homogenization for 30 s using a vortex blender, 7 mL of trichloromethane

Table 2. Typical Vitamin E and Carotenoid Contents of Selected Foods (*2*, *3*)

contributors of vitamin E[a]	α-tocopherol (mg/100 g)	γ-tocopherol (mg/100 g)	contributors of β-carotene[b]	β-carotene (mg/100 g)	contributors of lycopene[b]	lycopene (mg/100 g)	contributors of lutein[b]	lutein (mg/100 g)
wheat germ oil	155	50	carrot, raw	8.84	tomato sauce	15.92	spinach, cooked, boiled, drained	7.04
sunflower oil	61	2.70	carrot, canned	5.78	tomatoes (red, ripe, raw)	3.03	lettuce, romaine	2.64
hazelnut	26	1.90	spinach, cooked, boiled, drained	5.24	watermelon	4.87	green bean, canned	0.66
almonds	26	0.87	tomatoes (red, ripe, raw)	0.39				
wheat germ	21	ND[c]	tomato sauce	0.41				
lettuce	0.57	0.34						
Camembert cheese (45% fat)	0.50	ND						
apples, fresh	0.49	ND						
carrot	0.44	ND						
wheat bread (white bread)	0.40	0.30						
bananas, fresh	0.27	ND						
cow's milk (UHT)	0.09	ND						

[a] These foods are rich in vitamin E and count among the main dietary sources of vitamin E in the U.S. diet (*38*). [b] These foods are rich in the selected carotenoids and count among the main dietary sources of these carotenoids in Spain (*39*). [c] ND, not detected.

(containing 0.005% butylated hydroxy toluene as an antioxidant) was added and the sample was then homogenized for a further 30 s in the vortex blender. After 15 min rest, 7 mL of distilled water was added. After centrifugation (2000*g* for 10 min at room temperature), the lower phase containing more than 80% of the HLFMs was collected. The remaining HLFMs in the upper phase were extracted as follows: after addition of 5 mL of tetrahydrofuran, the mixture was vortexed for 30 s, and 5 mL of dichloromethane added. It was then vortexed for another 30 s, after which 3 mL of distilled water was added and the mixture was re-vortexed for a further 30 s. After centrifugation (2000*g* for 10 min at 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 200 μL of acetonitrile/dichloromethane (50/50; v/v).

Carotenoids were quantified by reverse-phase HPLC on a Waters system (Waters SA, Saint-Quentin-en-Yvelines, France). This system comprised a Waters 660 pump, a Waters 717 + cooled auto-sampler, and a Waters 996 UV−visible diode-array detector. Carotenoids were separated using a 150 × 4.6 nm RP C_{18}, 3-μm Nucleosil (Interchim, Montluçon, France). The mobile phase was an isocratic acetonitrile−dichloromethane−methanol (containing 50 mmol/L 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) in comparison with pure standards.

Vitamin E (α- and γ-tocopherol) was quantified by reverse-phase HPLC on the same system as described for carotenoid detection above. Vitamins were detected at 292 nm and identified by retention time and spectral analysis (from 200 to 400 nm) in comparison with pure standards. The column was a C18-Nucleosil (250 × 4.6 mm, 5 μm), and the mobile phase was 100% methanol. *all-rac*-α-Tocopherol acetate was used as internal standard.

All the solvents used for HPLC mobile phases were HPLC grade obtained from SDS (Peypin, France). Quantification was performed using Waters Millennium 32 software (version 3.05.01).

Data Used To Validate the Model. In order to validate the model, we assessed whether there was a relationship between the model-derived carotenoid bioaccessibility values and carotenoid bioaccessibility values measured in humans (*23*) and carotenoid bioavailability data obtained in human studies.

Calculations and Statistics. Bioaccessibility was defined as the percentage of meal-derived HLFM recovered in the micellar fraction after *in vitro* digestion in relation to the amount of meal-derived HLFM measured in the digestive medium just before pH was adjusted to 4.0. Results are expressed as means ± SEM. The correlation coefficients and their probability levels were obtained from linear regression analyses. Differences between means were assessed using the Student's t-test. *P* values <0.05 were considered significant. Statistical comparisons were performed using Statview software version 5.0 (SAS Institute Inc., Cary, NC).

RESULTS

Properties of the *in Vitro* Digestion Model. *Reproducibility*. Coefficient of variation (CV, not shown) as well as standard deviations (SD) observed showed that the model is reproducible.

Statistical Power. Since the number of experiments required to find a significant difference ($P < 0.05$, with a two-tailed unpaired t-test) in bioaccessibility between two matrices is SD-dependent, we calculated that pentaplicates were necessary to find a significant 5.2% difference in bioaccessibility with SD at 2%.

Detection Threshold. The detection threshold of the model is governed by several parameters: HLFM concentration in the food/supplement to be tested, the bioaccessibility of the HLFM, the volume of aqueous micellar phase extracted to measure HLFM, and the HPLC detection threshold. Given that (1) for practical reasons, extractions are usually made on 2 mL micelle samples, (2) the total volume of micellar fraction was 53 mL, (3) median bioaccessibility values for lycopene, β-carotene, lutein, and tocopherol in various foods were around 1, 5, 14, and 23%, respectively, and (4) the HPLC detection threshold was around 2 ng of carotenoid and 10 ng of vitamin E per HPLC injection, we therefore calculated that the amount of HLFM added to the test meal (and therefore contained in the tested food matrix or supplement) should be higher than 5.3, 1.1, 0.4, and 1.2 μg for lycopene, β-carotene, lutein, and α-tocopherol, respectively.

Table 3. Comparison of Bioaccessibility Values Measured *in Vivo* in a Published Study[a] and with the *in Vitro* Model in the Current Study

carotenoid in food	% carotenoids recovered in micelles *in vivo* (mean ± SEM)	% carotenoids recovered in micelles *in vitro* (mean ± SEM)
β-carotene in carrot puree	5.01 ± 0.72	4.39 ± 0.18
α-carotene in carrot puree	4.69 ± 0.67	8.88 ± 0.45
lycopene in tomato puree	2.23 ± 0.64	1.11 ± 0.17
lutein in spinach	7.72 ± 2.24	37.55 ± 4.06
β-carotene in spinach	2.97 ± 1.02	2.43 ± 0.20

[a] As measured in the human duodenum following carotenoid-rich meals (*23*). There was a significant relationship ($r = 0.90$, $P = 0.038$) between means bioaccessibility values measured *in vivo* and *in vitro*.

Correlations between Bioaccessibility Values Obtained with the *in Vitro* Model and Bioaccessibility Values Measured in a Published Human Study. Tyssandier et al. (*23*) estimated the *in vivo* bioaccessibility of carotenoids by measuring the percentage of carotenoids recovered in micellar phase from human duodenum during digestion of a carotenoid-rich meal. As shown in **Table 3**, the bioaccessibility values measured with the *in vitro* model in the current study were in the same range than to those measured *in vivo* in a published study, with the exception of spinach lutein bioaccessibility which was about 5-fold higher *in vitro* than *in vivo*. Nevertheless, both studies identified lycopene from tomato puree as the least bioaccessible carotenoid and lutein from spinach as the most bioaccessible carotenoid. Overall, the two sets of data correlated ($r = 0.90$, $P = 0.038$).

Comparison between Bioaccessibility Values Obtained with the *in Vitro* Model and Bioavailability Values Measured in Published Human Studies. Although particularly wide ranges of bioavailability ratios have been measured in human studies, there was a significant relationship ($r = 0.98$, $P < 0.0001$) between bioaccessibility ratios measured *in vitro* and the mean bioavailability ratios measured in groups of healthy human (**Table 4**).

Bioaccessibility of Carotenoids from Their Main Dietary Sources. Carotenoid bioaccessibility, measured with the *in vitro* digestion model, from different food matrices is shown in **Table 5**. Carotenoid bioaccessibility ranged from 1.6 to 14.5 for α-carotene, from 0.1 to 17.5 for β-carotene, from 0.1 to 1.6 for lycopene, and from 37.6 to 59.4 for lutein. The bioaccessibility of different carotenoid species present together in the same food source was highly variable. For example, in pumpkin, α-carotene was about 5-fold more bioaccessible than β-carotene, but this difference was not similar in all foods. In tomato sauce, lutein was about 9-fold more bioaccessible than β-carotene. On the whole, lutein was always more bioaccessible than the other carotenoids. Bioaccessibility also depended on the food matrix in which the carotenoid was embedded. For example, β-carotene bioaccessibility ranged from less than 0.1% in crude tomato and watermelon to around 17% in boiled spinach. Furthermore, lycopene bioaccessibility ranged from 0.1% in crude tomato to 1.6% in processed tomatoes. Food processing also affected carotenoid bioaccessibility. The bioaccessibility of β-carotene

Table 4. Relative Bioavailability Values Reported in Published Human Studies and Relative Bioaccessibility Values Measured in the Current Study with the *in Vitro* Digestion Model

study reference	carotenoid studied	matrix compared	mean bioav ratio[a]	bioav ratio (range)[b]	bioacc ratio[c]
Richelle et al., 2002 (*40*)	lycopene	lactolycopene[d] vs tomato puree	1.23	0.8–1.8	3.02
Bohm et al., 1999 (*41*)	lycopene	tomato juice vs raw tomatoes	1.43	0–∞	1.44
Bohm et al., 1999 (*41*)	lycopene	tomato juice vs lycopene oleoresin	0.67	0–12.5	0.13
Paetau et al., 1998 (*42*)	lycopene	tomato juice vs lycopene oleoresin	1	0.6–1.7	0.13
Porrini et al., 1998 (*43*)	lycopene	tomato puree vs raw tomatoes	1.5	0.7–2.1	1.44
Reboul et al., 2004 (*44*)	lycopene	skin-enriched vs classic tomato puree	1.54	0.9–2.6	1.29
Van Lieshout et al., 2003 (*45*)	β-carotene	pumpkin vs spinach	1.7	0.9–3.1	0.34
Thurmann et al., 2002 (*46*)	β-carotene	supplement[e] vs carrot juice (6 mg)	7.6	3.6–41.1	22.09
Livny et al., 2003 (*47*)	β-carotene	cooked vs raw carrots	1.54	1.2–2.1	1.11
Riso et al., 2003 (*48*)	lutein	supplement[f] vs spinach	2.41	0.7–10.5	3.61

[a] Mean bioavailability ratio: Ratios of the mean carotenoid plasma concentration or the mean carotenoid area-under-the-curve (AUC) of the postprandial response curve measured after intake of a first carotenoid-rich matrix to that measured after intake of a second carotenoid-rich matrix. A ratio of 1.23 means that lycopene from lactolycopene was 1.23-fold more bioavailable than lycopene from tomato puree. [b] Minimal and maximal ratios (minimal ratio = lower plasma concentration or AUC after ingestion of the first matrix/higher corresponding concentration or AUC after ingestion of the second matrix; maximal ratio = higher concentration or AUC after the first matrix/lower concentration or AUC after the second matrix). [c] Bioaccessibility ratio: amount of carotenoids recovered in the micellar phase after *in vitro* digestion of the first matrix compared to that recovered in the micellar phase after *in vitro* digestion of the second matrix. [d] Lactolycopene is a formulation in which lycopene is entrapped with whey proteins (*40*). [e] β-Carotene 30% FS from DSM in the *in vitro* digestion model. [f] Lutein 20% FS from DSM in the *in vitro* digestion model.

Table 5. Bioaccessibility of Carotenoids from Foods[a]

	α-carotene	β-carotene	lycopene	lutein
carrot, canned	3.36 ± 0.42[b]	2.68 ± 0.10	–[c]	53.83 ± 3.11
carrot juice	14.53 ± 2.58	14.14 ± 2.69	–	–
carrot puree	8.88 ± 0.45	4.39 ± 0.35	–	–
carrot, raw	1.62 ± 0.19	2.56 ± 0.24	–	43.88 ± 1.19
green peas	–	–	–	59.43 ± 5.07
pumpkin	6.71 ± 0.63	1.30 ± 0.22	–	–
spinach (boiled)	–	17.45 ± 1.99	–	47.82 ± 2.60
spinach (leaves)	–	2.43 ± 0.20	–	37.55 ± 4.06
spinach (minced)	–	5.20 ± 0.53	–	48.10 ± 0.26
tomato (raw)	–	<0.1	0.10 ± 0.05	51.92 ± 6.20
tomato (processed)	–	5.97 ± 0.81	1.60 ± 0.22	57.36 ± 6.61
watermelon (crude)	–	<0.1	0.35 ± 0.10	48.64 ± 5.90

[a] Measured with the *in vitro* digestion model in the current study. One to four grams of carotenoid-rich food was incubated in the digestive medium, and the bioaccessibility was measured as the percentage of carotenoid transferred into the mixed micellar fraction. [b] Mean ± SEM of at least three measurements. [c] Not detected or not measured.

Table 6. Bioaccessibility of Vitamin E from Foods[a]

	α-tocopherol	γ-tocopherol
almonds	14.18 ± 4.93[b]	19.78 ± 6.93
apples, fresh	0.47 ± 0.13	6.54 ± 2.60*
bananas, fresh	98.80 ± 1.80	6.88 ± 2.64*
bread (white wheat bread)	99.62 ± 11.30	8.36 ± 4.60*
cereals (wheat germ)	53.29 ± 7.85	47.50 ± 6.63
cheese (Camembert, 45% fat)	28.67 ± 6.22	59.09 ± 9.17
hazelnut	10.49 ± 5.29	24.52 ± 9.92
lettuce, romaine	101.31 ± 1.25	27.13 ± 6.73*
milk (cow, UHT)	21.95 ± 1.60	6.76 ± 2.59*

[a] Measured with the *in vitro* digestion model in the current study. One to four grams of vitamin E-rich food was incubated in the digestive medium and the bioaccessibility was measured as the percentage of vitamin E transferred into the mixed micellar fraction. An asterisk indicates that α- and γ-tocopherol bioaccessibility values were significantly different ($P < 0.05$). [b] Mean ± SEM of at least three measurements.

from raw carrots was 2.56% compared to 14.1% for carrot juice. In summary, carrot juice and processed tomato were the most bioaccessible sources for β-carotene and lycopene, respectively. Conversely, both β-carotene and lycopene exhibited very low bioaccessibility from crude tomato and watermelon sources. Lutein bioaccessibility was close in all tested foods (from 43.9% in raw carrots to 53.8% in canned carrots).

Bioaccessibility of Vitamin E from Foods. As shown in **Table 6**, vitamin E bioaccessibility, measured with the *in vitro* model, was extremely variable, ranging from 0.47% (from apple α-tocopherol) to almost 100% (banana, white bread, and lettuce α-tocopherol). With the exception of apple as source, α-tocopherol showed similar bioaccessibility (when sourced from almonds, wheat germ, cheese, and hazelnut) or higher bioaccessibility (when sourced from bananas, bread, lettuce, and milk) than γ-tocopherol.

DISCUSSION

The first objective of this study was to optimize the *in vitro* model proposed by Garrett et al. (*4*) in order to more closely mimic *in vivo* absorption and then to check whether bioaccessibility values obtained with this model correlate with those observed in vivo and whether bioaccessibility values are related with mean bioavailability values observed in human studies. The significant positive relationship between percentage of carotenoids transferred into micelles in the *in vitro* model and percent of carotenoids transferred into micelles observed *in vivo* (*23*) suggests that the model is suitable for predicting the bioaccessibility of carotenoids from foods. The problem is the very high percentage (37.55%) of lutein transferred into mixed micelles *in vitro* that does not fit with the percentage reported in the human study (7.7%). In fact, a close look at the bioaccessibility data obtained in the human study which was performed by our team (*23*) shows that, conversely to spinach lutein, tomato lutein as well as carrot lutein appeared very bioaccessible (37 and 26%, respectively, data not published). Furthermore, lutein bioaccessibility measured with the *in vitro* model was always higher than 37%, all tested food sources included (**Table 5**). It is therefore probable that the bioaccessibility of spinach lutein was underestimated in the human study, likely because of interference between chlorophylls and lutein for lutein measurement (deduced after a close look at the HPLC chromatograms). The positive correlation between the bioaccessibility ratios measured with the *in vitro* model and the bioavailability ratios measured in human studies suggests that the model can give an estimate of the mean bioavailability of carotenoid food sources in a healthy population. Nevertheless, there were some strong differences between some bioaccessibility ratios obtained with the *in vitro* model and mean bioavailability ratios measured *in vivo*. These differences can

be explained by several factors. First, it should be kept in mind that no model is ideal, and although we are naturally more confident in *in vivo* data, there is for example no proof that postprandial chylomicron response gives an absolute reflection of long-term bioavailability. Second, bioavailability ratios are mean ratios which are not therefore calculated with values from individual subjects, and the bioavailability ratios reported here were generally high, suggesting that the means calculated from human studies with a relatively small number of subjects may not always be representative of the means of the whole population. This probably stems from the high interindividual variability in carotenoid response (*1*, *27*), since populations consist of low and high absorbers. Third, some studies estimated bioavailability by the increase in plasma concentrations of carotenoids rather than by area under the curve of the postprandial chylomicron carotenoid response. However, it is well-known that plasma carotenoid concentrations are affected not only by absorption efficiency but also by metabolism and clearance rate. Finally, bioaccessibility is not the only factor which affects absorption (*1*), and it is possible that a highly bioaccessible carotenoid has a low absorption efficiency, since some factors associated with the food matrix may inhibit absorption. The underlying mechanism remains unknown, but as we recently found that carotenoid absorption is, at least partly, mediated by membrane transporter(s) (*28*), we hypothesize that effectors such as phytosterols and (or) phospholipids, which are associated with the carotenoid-rich vegetable matrix, may modulate the activity of this transporter.

Although there is a lack of human study data to verify whether the *in vitro* model can predict vitamin E bioavailability, there is no reason to believe that it is not as efficient for this fat-soluble vitamin as it is for the fat-soluble carotenoids. Indeed, vitamin E and carotenoids are assumed to share a similar fate in the duodenum. Furthermore, the fact that the bioaccessibilities measured for vitamin E were higher than those measured for lycopene and β-carotene is in good agreement with the assumption that vitamin E is better absorbed than carotenoids.

The second and main objective of this study was to use the *in vitro* model to measure bioaccessibility of carotenoids and vitamin E from foods and therefore to estimate the most appropriate sources of these micronutrients. A comprehensive table giving the relative bioaccessibility of these microconstituents from their dietary sources would be very useful to nutritionists. The fact that the bioaccessibility of carotenoids and vitamin E varied strongly between different food sources (**Tables 5** and **6**) confirms that food matrix has a marked effect on HLFM bioavailability (*1*). This can be explained by different locations of HLFM in foods, different physicochemical states of HLFM in different foods, and different kinds and amounts of absorption effectors, i.e., fibers (*29*), fats (*30*), and phytosterols (*31*). The higher bioaccessibility of lutein compared to β-carotene and lycopene whatever the food source is in agreement with previous data (*32*, *33*). We suggest that this is due to the lower lipophilicity of lutein leading to a higher solubility of this carotenoid in micelles. The beneficial effect of food processing on carotenoid bioaccessibility is in full agreement with *in vivo* studies on carotenoid bioavailability (*34*, *35*) and confirms that eating processed vegetables improves carotenoid bioavailability. There is only scarce data on vitamin E bioavailability from different food matrices, and it is only assumed that bioavailability from seeds is low as these sources are not readily digested in the human GI tract (*36*). It has also been found that vitamin E bioavailability was lower from a supplement than from cereals (*37*). To the best of our knowledge, **Table 6** provides the first comparison of vitamin E bioaccessibilities between its main dietary sources. The results obtained suggest that vitamin E bioaccessibility is highly variable between dietary sources. This important observation requires further experiments to be conclusively demonstrated.

In conclusion, we have shown that an *in vitro* digestion model to measure the bioaccessibility of carotenoids and vitamin E from foods can be a valuable tool to identify the most appropriate sources of carotenoids and, likely, vitamin E. Further human studies are required to confirm whether this model is able to predict the bioavailability of not only carotenoids and vitamin E but also other fat soluble micronutrients, such as vitamin D and K.

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LITERATURE CITED

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