Combining the Dynamic TNO-Gastrointestinal Tract System with a Caco-2 Cell Culture Model: Application to the Assessment of Lycopene and α-Tocopherol Bioavailability from a Whole Food

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To exert their health effect, phytochemicals such as carotenoids and vitamin E have to be bioavailable. We investigated the digestive stability and intestinal absorption of lycopene and α-tocopherol from a whole food containing red tomatoes and sunflower oil using, for the first time, the dynamic gastrointestinal system TNO gastrointestinal tract model (TIM) coupled with Caco-2 cells. Digestive samples were added to Caco-2 cells after appropriate ultracentrifugation, filtration, and dilution. α-Tocopherol was stable during digestion in the TIM, whereas a 25% loss was observed for lycopene. The absorption of both compounds was curvilinear, bidirectional, and concentration-dependent. The percentages of α-tocopherol absorbed, but not that of lycopene, were lower with digestas compared to those with pure compounds, suggesting competition for absorption with other components of the test meal. According to in vivo data, a lower bioavailability was found for lycopene compared to that for α-tocopherol. These results support the usefulness of this in vitro approach for estimating the bioavailability of active compounds from food.

INTRODUCTION

Epidemiological studies have suggested that the consumption of phytochemicals such as the red pigmented carotenoid lycopene and vitamin E reduces the risk of several human chronic diseases, such as cancers or cardiovascular diseases (*1*, *2*). It is assumed that they act through their specific biological activities such as free radical scavenging, lipid peroxidation inhibition, and anticarcinogenic properties. In order to mediate their health effect, these components should be bioavailable, i.e., absorbed and delivered to the target tissues for storage or utilization. Given the limitations in the use of animal or human subjects, simple in vitro digestion models, sometimes coupled with intestinal cells in culture, have been developed for assessing carotenoids and vitamin E bioavailability (*3*, *4*). These systems have been widely used to study food-related factors (food matrix and food processing) affecting the bioavailability of these components (*5*–*8*), with a high predictive value (*5*, *6*, *8*). Nevertheless, they are monocompartmental and static and are not representative of the continuously changing variables during passage through the stomach and small intestine.

Alternatively, the TNO gastrointestinal tract model (TIM) is a dynamic computer-controlled in vitro system that closely mimics the physiological processes occurring within the lumen of the stomach and small intestine of humans (*9*). Contrary to previously mentioned static models, the TIM system reproduces (i) the three compartments of the human small intestine, (ii) chyme transit from one digestive compartment to the next, (iii) pH change during gastric digestion and from the duodenum to ileum, (iv) sequential arrival of digestive secretions, and (v) passive absorption of small molecules and water. It has been validated by microbial, pharmaceutical, and nutritional studies (*10*, *11*). In particular, the TIM system has shown its usefulness in studying the digestive stability of carotenoids from different food matrixes throughout the gastrointestinal tract (*12*). Nevertheless, current limitations of the model include the absence of a cellular system and active absorption of nutrients not being reproduced. Human intestinal cells in culture, such as Caco-2 cells, provide a physiologically relevant model for studying the absorption and metabolism of nutrients and drugs (*13*).

The objectives of this study were (i) to develop a Caco-2 cell culture model coupled with the TIM system and (ii) to assess the luminal stability and intestinal absorption of lycopene and vitamin E (α-tocopherol) from a whole food containing red tomatoes and sunflower oil.

MATERIALS AND METHODS

Chemicals. Mineral salts, sodium bicarbonate, chlorhydric acid, pepsin (porcine), pancreatin (porcine), trypsin (porcine), bile extract

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Table 1. Parameters of Gastrointestinal Digestion in the TIM When Simulating Digestive Conditions of a Healthy Adult after Intake of a Solid Meal

	gastric compartment	duodenal compartment	jejunal compartment	ileal compartment
volume	300 mL (at initial time)	30 mL	130 mL	135 mL
pH/time (min)	6/0	maintained at 6.4	maintained at 6.9	maintained at 7.2
	5.7/15			
	4.5/45			
	2.9/90			
	2.3/ 120			
	1.8/ 240			
	1.6/ 300			
secretion	0.25 mL/min of pepsin (2072 IU/mL)	0.5 mL/min of bile salts (4% during the first 30 min of digestion then 2%)	0.25 mL/min of NaHCO$_3$ 1 M if necessary	0.25 mL/min of NaHCO$_3$ 1 M if necessary
	0.25 mL/min of lipase (250.5 IU/mL)	0.25 mL/min of pancreatic juice (10^3 USP/mL)		
	0.25 mL/min of HCl 1.5 M if necessary	0.25 mL/min of intestinal electrolyte solution		
		0.25 mL/min of NaHCO$_3$ 1 M if necessary		
		23600 IU of trypsin (at the beginning of digestion)		
$t_{1/2}$[a]	85 min			250 min
β coefficient[a]	1.8			2.5

[a] A power exponential equation ($f = 1 - 2^{-(t/t_{1/2})^\beta}$ where f represents the fraction of meal delivered, t the time of delivery, $t_{1/2}$ the half-time of delivery, and β a coefficient describing the shape of the curve) is used for the computer control of gastric and ileal deliveries (*15*).

(porcine), protease inhibitor tablets, hexane, Tween 80, α-tocopherol, α-tocoacetate, and lycopene were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, vitamins, nonessential amino acids, L-glutamine, antibiotic solution (penicillin 10^3 IU/mL, streptomycin 10 mg/mL, and amphotericin B 25 μg/mL), phosphate buffered saline (PBS), trypsin-EDTA, acetonitrile, ammonium acetate, dichloromethane, and methanol were purchased from Fisher Bioblock (Strasbourg, France). Recombinant human lipase was supplied by Amano Pharmaceuticals (Nagoya, Japan), and echinenone was purchased from DSM (Heerlen, The Netherlands).

Preparation of the Test Meal. The test meal was designed to contain all of the macronutrients present in a typical western diet and was adapted from that used by Tyssandier et al. in human studies (*14*). It was made of 60 g of cooked egg white, 50 g of cooked pasta, 2.5 g of soy lecithin (Les 3 chênes, Villechêne, France), 20 g of sunflower oil, and 100 g of double concentrated puree of red tomato (CTCPA, Aix-en-Provence, France). All of the ingredients (except soy lecithin) were purchased from a local store. The volume of the meal was adjusted to 300 mL with mineral water (Volvic, France), before homogenizing for 20 min with an Ultra Turrax system (T25, IKA, Werke, Staufen, Germany), set at 24000 rpm.

In Vitro Digestion. The in vitro digestion protocol was performed using the dynamic TIM system (TNO, Zeist, The Netherlands) which consists of four successive compartments simulating the stomach, duodenum, jejunum, and ileum. The main parameters of digestion, such as pH, body temperature, peristaltic mixing and transport, gastric, biliary, and pancreatic secretions, and passive absorption of small molecules and water are reproduced as accurately as possible (*9*). This system has been designed to accept parameters and data from in vivo studies on human volunteers. In the present study, the TIM was programmed to reproduce the digestion of a solid meal in a healthy human adult (**Table 1**). As the aim of the current work was to couple the TIM system with Caco-2 cells, passive absorption of digestion products was not reproduced (the hollow fibers and the dialysis pump were disconnected). To prevent photodecomposition of lycopene and α-tocopherol, all of the digestive compartments and collection vessels were wrapped in tinfoil. The intestinal compartments were maintained under nitrogen flow to limit phytochemical oxidation and reproduce the luminal conditions of oxygenation found in vivo. The ileal deliveries were collected on ice and pooled at 0−60, 60−120, 120−180, 180−240, and 240−300 min. The volumes were measured, and aliquots were taken for each period. The total duration of the digestion was 300 min (n = 3). All samples were stored at −20 °C prior to analysis or addition to intestinal cells in culture.

Cell Culture. The Caco-2 human colon cancer cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD) at passage 15. Stock cultures were maintained in DMEM with 4.5 g/L of glucose supplemented with 20% fetal bovine serum, 2% vitamins, 2% nonessential amino acids, 2% L-glutamine, and 2% of an antibiotic solution, at 37 °C in a humidified atmosphere of air/CO$_2$ (95:5, v/v). The medium was changed every 3 days. Cells were used for experiments at passages 22−40. Plates of culture 6 wells or Transwell polycarbonate cell culture supports were seeded with 5 × 10^5 cells per well, and the medium was changed every day. All experiments were carried out 11 to 14 days after initial seeding when Caco-2 cells exhibited maximum differentiation (*13*). Before each experiment, the medium was removed, and the cell monolayer was washed three times with 1 mL of PBS.

Cellular Toxicity of Digestive Secretions and Digestas. Cells were exposed during 6 h to 1 mL of each digestive secretion from the TIM (intestinal electrolyte solution, 4% bile salts, 10^3 USP/mL pancreatic juice, and 26300 UI/mL trypsin) or to 1 mL of the digestive samples (ileal deliveries pooled on 120−240 min). The viability of the cells was evaluated by trypan blue exclusion (final concentration of 0.1%). When Transwell cell culture supports were used, the transepithelial electrical resistance (TEER) was also measured (electrode STX2, EVOMX World Precision Instruments, Sarasota, FL, USA). The influence of sample dilution (1:2, 1:4, 1:8, 1:16, and 1:32 v/v in DMEM), ultracentrifugation (167000g, 35 min, 4 °C), filtration (0.45 μm), and/or addition of antiproteases on the cell viability was assessed.

Absorption of Lycopene and α-Tocopherol by Caco-2 cells. We developed two model designs to assess the absorption by Caco-2 cells of lycopene and α-tocopherol present either in synthetic micelles (design 1, pure compounds) or in micelles elaborated during in vitro digestion by the TIM of the whole food (design 2, digestas).

Design 1 (*Pure Compounds*). Lycopene or α-tocopherol was first dissolved in acetone using a nonionic surfactant Tween 80 (*16*, *17*) at a final concentration of 0.1%. The solvent was evaporated, and the dried residue was solubilized in DMEM. Two concentrations of lycopene (0.4 and 1.4 μg/mL) or α-tocopherol (0.9 and 2.4 μg/mL) were tested. One milliliter of each solution was added under yellow light to the apical or basolateral chamber of the insert containing the cells. Cells were incubated at 37 °C during 0.5, 1, 3, or 6 h (n = 3 for each time point). As a measure of cell viability and monolayer integrity, TEER was recorded throughout incubation. Experiments were stopped at indicated times by removing the treatment medium and washing the cells three times with PBS supplemented with 5 mM of sodium taurocholate. Washed cells were harvested and stored at −80 °C with the basolateral medium (when synthetic micelles were added to the apical chamber) or with the apical medium (when they were added to the basolateral chamber) containing 10% (v/v) methanol.

Design 2 (*Digestas*). Digestive samples (ileal deliveries pooled on 120−240 min) were ultracentrifuged (167000g, 35 min, 4 °C). The aqueous fraction containing mixed micelles was collected, filtered (0.45 μm), and diluted 1:4 v/v with DMEM before addition to the apical or basolateral chamber of the Caco-2 cells culture. Cells were then incubated and treated as described above (n = 3). Control experiments without cells were also

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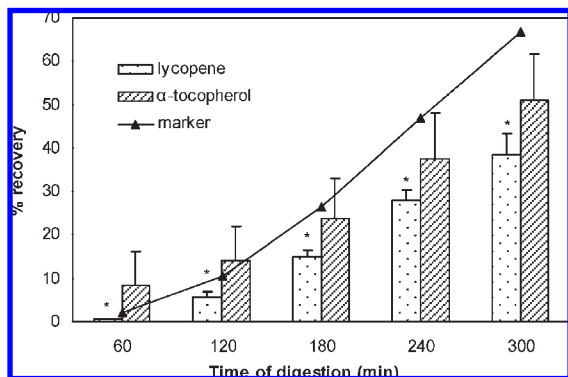
Figure 1. Recovery profiles of lycopene and α-tocopherol in the ileal deliveries of the TIM after in vitro digestion of a whole food containing red tomatoes and sunflower oil. The cumulative ileal deliveries of the phytochemicals and that of a marker transit, blue dextran, are represented. Values are expressed as mean $\pm$ SD (n = 3) percentage recovery. *Significantly different from the marker transit ($p < 0.05$).

conducted to evaluate the stability of lycopene and α-tocopherol in the culture medium.

Extraction and HPLC Analysis. Lycopene and α-tocopherol concentrations in the test meal, the ileal deliveries, and the samples collected during the absorption experiments were determined. Before extraction, the cell homogenates (with basolateral or apical medium) were sonicated for 5 min at room temperature. Lycopene and α-tocopherol were extracted from 500 μL of samples under yellow light, as previously described (*14*). Echinenone (0.2 μg/mL) and α-tocoacetate (20 μg/mL) were used as internal standards. Extracts were dried under nitrogen and dissolved in 200 μL methanol/dichloromethane (65/35, v/v) before injection into an HPLC apparatus. Lycopene and α-tocopherol were analyzed by reverse-phase HPLC using a Waters system (Saint-Quentin en Yvelines, France) equipped with a cooled autosampler injector and a UV−visible diode array detector system. Separation was carried out using two columns in a series: a Nucleosil C18 (150 × 4.6 mm, 3 μm) followed by a Vydac TP54 (250 × 4.6 mm, 5 μm) purchased from Interchim (Montluçon, France). The mobile phase was acetonitrile/methanol (containing 50 mmol/L ammonium acetate)/dichloromethane/water mixture (70/15/10/5, v/v/v/v). The flow rate was isocratic (2 mL/min). Lycopene and α-tocopherol were detected at 450 and 292 nm, respectively, and identified by retention time and spectral analysis in comparison with pure standards.

Calculations and Statistical Analysis of Data. The luminal stability of lycopene and α-tocopherol was evaluated by calculating the percentage recovery (amounts in digestas/amounts in test meal × 100). The recovery profiles obtained in the ileal deliveries were compared to that of a nonabsorbable marker, blue dextran, as previously described (*9*). Values are given as mean $\pm$ SD (3 independent replicates). Differences between the recovery percentages of lycopene or α-tocopherol and that of the transit marker were tested by ANOVA with repeated measure analysis followed by a post hoc test. Differences between cell viability and percentages of absorption were assessed by ANOVA. All analyses were performed using the SAS 9.1 software (SAS Institute, Inc., Cary, NC). A probability level of $p < 0.05$ was considered to be statistically different.

RESULTS

Digestive Stability of Lycopene and α-Tocopherol in the TIM. The concentrations of lycopene and α-tocopherol in the test meal were 31.1 ± 2.7 and 29.5 ± 2.0 mg/kg diet, respectively. The recovery profiles of these compounds in the ileal deliveries were compared to that of the transit marker (**Figure 1**). α-Tocopherol was stable during digestion in the TIM as its recovery profile in the ileal deliveries was not significantly different from that of the marker ($p > 0.05$). On the contrary, the recovery of lycopene was significantly lower than that of the marker throughout in vitro digestion ($p < 0.01$). At the end of the experiment (300 min), a

loss of around 25% of the initial lycopene was observed. The concentrations of lycopene and α-tocopherol in the ileal deliveries pooled on 120−240 min were 7.5 ± 0.6 μg/mL and 5.3 ± 1.7 μg/mL, respectively.

Cytotoxicity of Digestas and Digestive Secretions. Viability of Caco-2 cells after incubation with digestive secretions or digestas is shown in **Figure 2**. Electrolyte solution was not toxic (data not shown). On the contrary, pancreatic juice, bile salts, and trypsin (**Figure 2A−C**) were widely cytotoxic when not diluted (cell viability of approximately 5%). Ultracentrifugation (U) of samples allowed a significant increase in cell viability compared to that of the control, from the dilution 1:2 for pancreatic juice ($15 \pm 2\%$ vs $5 \pm 1\%$, $p = 0.001$) and from the dilution 1:4 for bile salts ($91 \pm 1\%$ vs $45 \pm 2\%$, $p < 0.001$) and trypsin ($60 \pm 2\%$ vs $45 \pm 2\%$, $p = 0.001$). The cell viability was more improved by an additional filtration (U + F). About 100% viability was reached from the 1:4 dilution when antiproteases (AP) were added to the digestive secretions. Whatever the tested treatment, the cytotoxicity of the digestive secretions disappeared from the dilution 1:8. The ileal deliveries were highly toxic when they were added to the cells pure or diluted at 1:2, with a cell viability less than 5% (**Figure 2D**). Higher dilution rates in DMEM allowed an improvement of the cell viability, but the cellular monolayer was intact only for the dilution 1:32 ($96 \pm 2\%$ of viable cells). None of the treatment tested (ultracentrifugation, filtration, and/or addition of antiproteases) led to a marked increase in viability compared to that of the control.

When the cells were cultured on wells (**Figure 3A**), the cell viability increased with the dilution rate from approximately 40% (1:8) to 100% (1:32). On the contrary, when the cells were cultured on inserts (**Figure 3B**), no cell mortality was observed from the dilution 1:8 whatever the treatment (filtration and/or ultracentrifugation). At dilution 1:4, while very low cellular survival rates were observed on wells (around 10%), the use of inserts led to a cell viability ranging from $25 \pm 4\%$ (control) to $95 \pm 1\%$ (U + F). When the cells were cultured on inserts, ultracentrifugation and filtration (U + F) allowed a significant increase in the percentage of viable cells, compared to ultracentrifugation alone (U) ($95 \pm 1\%$ vs $80 \pm 2\%$, $p < 0.001$).

Absorption of Lycopene and α-Tocopherol by Caco-2 Cells. Throughout absorption assays, TEER was stabilized around its initial value, i.e., approximately 175 Ω/cm^2. Control experiments without cells showed no degradation of lycopene and α-tocopherol for 6 h.

Time kinetics of α-tocopherol absorption by the Caco-2 monolayer are shown in **Figure 4**. When α-tocopherol was added to the apical chamber (**Figure 4A**), it was taken up in a time-dependent manner by the Caco-2 monolayer and could be detected as soon as 30 min in the basocellular medium. Absorption of α-tocopherol reached a saturated level after 3 h with digestas ($p > 0.05$ for 6 h vs 3 h) but not with pure compounds. The percentage of α-tocopherol absorbed was dependent on the dose added to the apical medium ($p < 0.05$) and on its matrix (i.e., pure compound vs digestas, $p < 0.05$). After a 6 h incubation period, the percentages of α-tocopherol reached $26.7 \pm 4.9\%$, $36.0 \pm 0.1\%$, and $37.7 \pm 0.1\%$ for digestas, with the pure compound at 2.4 μg/mL and pure compound at 0.9 μg/mL, respectively. When α-tocopherol was added to the basolateral chamber (**Figure 4B**), similar kinetic patterns were observed but with lower amounts absorbed throughout experiments. At 6 h, the percentages of α-tocopherol reached $12.9 \pm 6.8\%$, $23.4 \pm 0.1\%$, and $25.0 \pm 0.1\%$ for digestas, with pure compound at 2.4 μg/mL and pure compound at 0.9 μg/mL, respectively.

Time kinetics of lycopene absorption by the Caco-2 monolayer are shown in **Figure 5**. As previously observed for α-tocopherol,
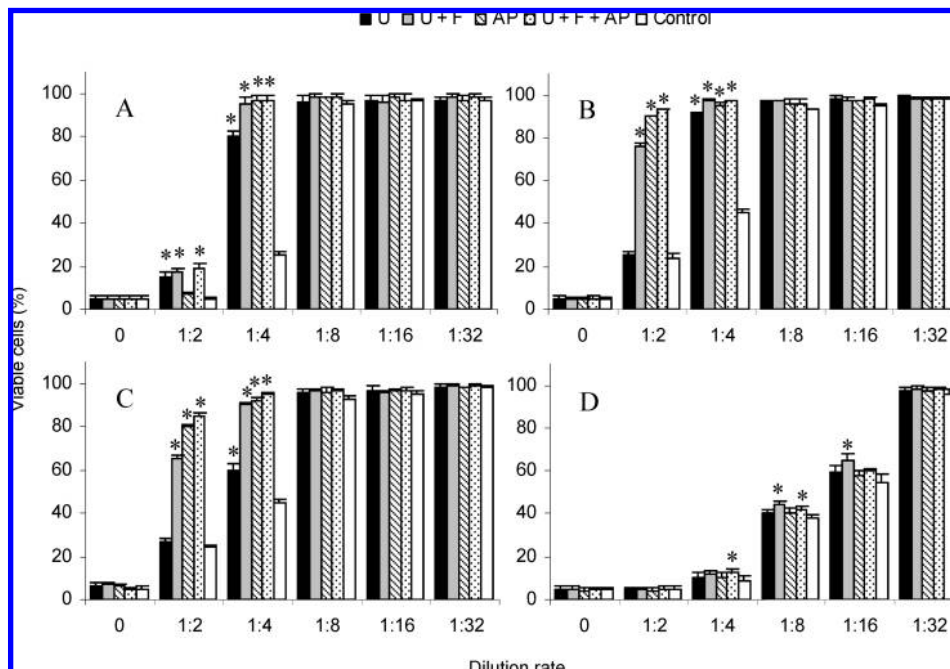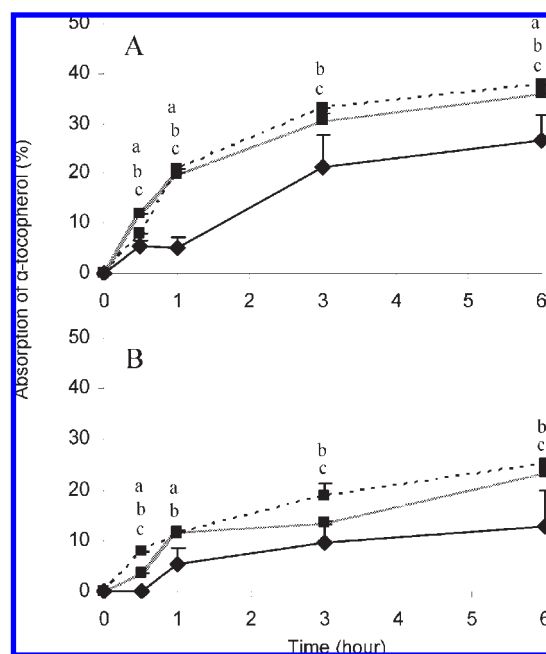
Figure 2. Influence of pancreatic juice (**A**), bile salts (**B**), trypsin (**C**), and ileal deliveries (**D**) on the viability of Caco-2 cells. The percentage of viable cells was determined by blue trypan staining after dilution of the samples in DMEM and treatment by ultracentrifugation (U), filtration (F), and/or addition of antiproteases (AP). The control samples were only diluted. Values are means $\pm$ SD (n = 3). *Significantly different from the control samples ($p < 0.05$).
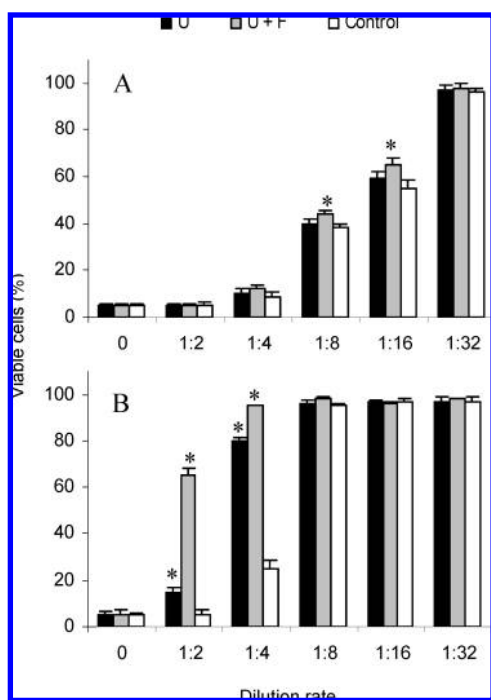
Figure 3. Influence of ileal deliveries on the viability on Caco-2 cells when cultured on wells (**A**) or inserts (**B**). The percentage of viable cells was determined by blue trypan staining after dilution of the samples in DMEM and treatment by ultracentrifugation (U), possibly followed by filtration (U + F). The control samples were only diluted. Values are means $\pm$ SD (n = 3).*Significantly different from the control samples ($p < 0.05$).
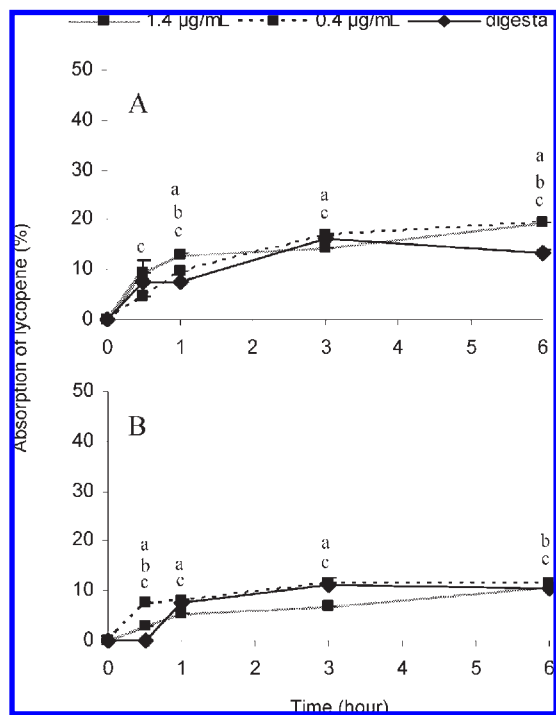

Figure 4. Time kinetics of α-tocopherol absorption by Caco-2 cells after deposit on the apical (**A**) or basolateral (**B**) side of the monolayer. Caco-2 cells were incubated with 1 mL of ultracentrifuged (167000g, 35 min, 4 °C), filtered (0.45 μm), and diluted (1:4) digestas or pure α-tocopherol at two concentrations (0.9 or 2.4 μg/mL). Values are means $\pm$ SD (n = 3). (a) Digestas significantly different from pure α-tocopherol at 2.4 μg/mL, (b) digestas significantly different from pure α-tocopherol at 0.9 μg/mL, and (c) α-tocopherol at 0.9 μg/mL significantly different from α-tocopherol at 2.4 μg/mL ($p < 0.05$).

when lycopene was added to the apical or basolateral chamber, it was taken up in a time-dependent manner by the Caco-2 mono-layer and could be detected as soon as 30 min in the opposite compartment. When lycopene was added to the apical chamber (**Figure 5A**), its absorption reached a saturated level after 3 h with

digestas but not with pure compounds ($p < 0.05$ for 6 h vs 3 h). The absorption profiles of lycopene were similar whatever the carotenoid matrix and concentration. When lycopene was added to the basolateral chamber (**Figure 5B**), this carotenoid reached a

Figure 5. Time kinetics of lycopene absorption by Caco-2 cells after deposit on the apical (**A**) or basolateral (**B**) side of the monolayer. Caco-2 cells were incubated with 1 mL of ultracentrifuged (167000g, 35 min, 4 °C), filtered (0.45 μm), and diluted (1:4) digestas or pure lycopene at two concentrations (0.4 or 1.4 μg/mL). Values are means $\pm$ SD (n = 3). (a) Digestas significantly different from pure lycopene at 1.4 μg/mL, (b) digestas significantly different from pure lycopene at 0.4 μg/mL, and (c) lycopene at 0.4 μg/mL significantly different from lycopene at 1.4 μg/mL ($p < 0.05$).

stable level after 3 h, except when the 1.4 μg/mL concentration was used. The percentage of pure lycopene absorbed was significantly higher when the lowest concentration was used ($p < 0.001$). Whatever the side of deposit, the amount of lycopene absorbed remained lower than that of α-tocopherol (after 6 h of incubation, from 10 to 20% for lycopene vs 15 to 35% for α-tocopherol).

DISCUSSION

Accurate prediction of phytochemical bioavailability from a whole food is a significant challenge. The present study was designed to assess the luminal stability and intestinal absorption of lycopene and α-tocopherol from a standard meal containing red tomatoes and sunflower oil by using the TIM system and a Caco-2 cell culture model. To closely mimic real conditions, these phytochemicals were bought in nutritional quantities (*18*, *19*).

Very few in vitro or in vivo studies have evaluated the digestive stability of lycopene or α-tocopherol. Recovery percentages of lycopene close to that found in our study (ca. 75%) were obtained by Failla et al. (*20*) after gastrointestinal digestion of gac fruit cooked with rice. A previous in vitro study in the TIM system showed that lycopene from red tomato was stable in the gastric and duodenal compartments but was degraded in the lowest parts of the small intestine (*12*). It appears that trans−cis isomerization of lycopene does not occur during digestion both in vitro (*12*, *20*) and in vivo (*14*). Nondetected metabolites (such as oxidation products) or degraded molecules of lycopene might thus be produced during small intestinal digestion, but no precise data could support this hypothesis. Regarding α-tocopherol, our

results are consistent with those of Granado et al. (*6*) and Granado-Lorencio et al. (*7*), who found no or only a slight degradation during in vitro gastric or duodenal digestion, and those of Borel et al. (*21*), who showed that this vitamin was not degraded in the human stomach.

Prior to absorption experiments, we assessed the cytotoxicity of digestas from the TIM on the Caco-2 monolayer and the influence on the cell viability of various treatments, such as sample dilution, ultracentrifugation, filtration, and/or addition of antiproteases, as previously suggested by Garrett et al. (*4*), Gangloff et al. (*22*), and Liu et al. (*23*). The experiments were conducted with the ileal deliveries pooled on 120−240 min where lycopene and α-tocopherol were found in the largest quantities. Our results point out a toxic effect of both digestive secretions and meals (*4*, *22*), as enzymatic inactivation by addition of antiproteases led to a decrease in the cytotoxicity of intestinal secretions but did not modify that of ileal deliveries. None of the tested parameters allowed us to find satisfactory conditions for the treatment of digestas since the only condition that led to an intact monolayer (dilution 1:32) was not compatible with the requirement of absorption assays (lycopene and α-tocopherol concentrations under the limit of quantification by HPLC). Additional toxicity assays were then conducted on polycarbonate inserts (*13*), which are supposed to improve the fixation and growth of the cells. Cell viability close to 100% was actually observed after 1:4 dilution, ultracentrifugation (167000g, 35 min, 4 °C), and filtration (0.45 μm) of the digestas, conditions that were selected for the following experiments of absorption.

The absorption assays were conducted on the Caco-2 human intestinal cell line, which has proved to be a useful model for studying the transport and metabolism of dietary compounds (*24*). The absorption of α-tocopherol and lycopene from a whole food or from pure compounds was investigated. The two concentrations of α-tocopherol (0.9 and 2.4 μg/mL) and lycopene (0.4 and 1.4 μg/mL) tested were chosen to be close to physiological doses and representative of the amounts found in the ileal deliveries of the TIM after appropriate treatments. Whatever the side of deposit, the amounts of α-tocopherol and lycopene absorbed increased curvilinearly, as previously observed by other authors for α-tocopherol (*16*, *25*) and carotenoids such as lutein or β-carotene (*23*, *24*). The saturable absorption of α-tocopherol and lycopene as well as their bidirectional passage through the monolayer strongly argue in favor of a protein-mediated absorption. Our results confirm those obtained in recent studies which suggest that the absorption of α-tocopherol (*25*) and lycopene (*26*) is partly due to a facilitated process mediated by a membrane transporter, the scavenger receptor class B type I (SR-BI). SR-BI transporters are mainly present on the apical surface of Caco-2 cells (*27*), which could explain the higher amounts absorbed when α-tocopherol or lycopene is added to the apical chamber compared to the basolateral one.

We further investigated the effect of α-tocopherol and lycopene concentrations and matrixes (digestas vs pure compounds) on their absorption. The percentages of pure compounds absorbed were significantly higher when the lowest concentrations were used. Similar results were obtained in humans for lycopene (*28*, *29*) and in rats or humans for α-tocopherol (*30*, *31*). A matrix effect was observed for α-tocopherol but not for lycopene. In addition to lycopene and α-tocopherol, the test meal provides other components that may change their behavior (other carotenoids or tocopherols, lecithins, etc.). In particular, it contains small concentrations of β-carotene, lutein, and zeaxanthin (*12*). Interactions between carotenoids during absorption have been demonstrated or suggested in many in vitro, animal or human studies (*32*). Particularly, interactions between β-carotene and

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lycopene have been observed in humans (*33*). The majority of literature reporting such interactions involved concentrations greater than that found in food. We can assume that such phenomena were not observed in this study because more physiological concentrations were used. The percentages of α-tocopherol absorbed were significantly lower with digestas compared to that with pure compounds. Other phytochemicals of the test meal known to be transported through the SR-BI may have competed with α-tocopherol for absorption. This is the case of carotenoids such as lycopene, β-carotene, or lutein (*34*, *35*) and other forms of vitamin E that were not dosed in this study, such as γ-tocopherol (*25*, *36*).

Combining the TIM system with Caco-2 cells, we found a lower bioavailability for lycopene compared to that for α-tocopherol. A high interindividual variability in their absorption or plasma level was reported by the literature. Nevertheless, in vitro (*24*) and in vivo data (*33*, *37*, *38*) agree to indicate that lycopene is poorly absorbed compared to other carotenoids during digestion, with percentages ranging from 2 to 10%. In what concerns α-tocopherol, higher percentages are reported with an intestinal absorption ranging from 20% to 80% in humans (*39−41*).

In conclusion, the present study provides the first assessment of lycopene and α-tocopherol bioavailability from a whole food using the dynamic gastrointestinal TIM system coupled with a Caco-2 cell model. Overall, in vitro results are consistent with in vivo observations supporting the potential applicability and predictive value of this in vitro approach to assess the bioavailability of bioactive compounds from food or supplements.

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