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Digestive Stability, Micellarization, and Uptake of β-Carotene Isomers by Caco-2 Human Intestinal Cells

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While isomeric profiles of carotenoids found in food often differ from those in body fluids and tissues, insights about the basis for these differences remain limited. We investigated the digestive stability, relative efficiency of micellarization, and cellular accumulation of trans and cis isomers of β-carotene (BC) using an in vitro digestion procedure coupled with human intestinal (Caco-2) cells. A meal containing applesauce, corn oil, and either water-soluble beadlets (WSB) or *Dunaliella salina* (DS) as a BC source was subjected to simulated gastric and small intestinal digestion. BC isomers were stable during digestion, and the efficiency of micellarization of *cis*-BC isomers exceeded that of all-*trans*-BC isomers. The cellular profile of carotenoids generally reflected that in micelles generated during digestion, and intracellular isomerization was minimal. These data suggest that cis isomers of BC are preferentially micellarized during digestion and transferred across the brush-border surface of the enterocyte from mixed micelles with similar efficiency as all-*trans*-BC at the concentrations of the carotenoids utilized in this study.

KEYWORDS: β-carotene; carotenoids; cis isomers; in vitro digestion; Caco-2 intestinal cells; HPLC; electrochemical detection

INTRODUCTION

Carotenoids are a family of hydrophobic pigments located in chloroplasts and chromoplasts of algae and plants. In addition to the more than 600 species that have been identified, carotenoids are known to exist as different geometric isomers. For example, the primary species of provitamin A carotenoid β-carotene (BC) in fresh foods is the all-trans isomer. However, appreciable amounts of 9-, 13-, and 15-cis isomers (**Figure 1**) may also be present in processed foods (*1*−*3*). All-*trans*- and 9-*cis*-BC are precursors for all-*trans*- and 9-*cis*-retinoic acid (RA), respectively. All-*trans*-RA preferentially binds to RA nuclear receptor (RAR), whereas 9-*cis*-RA is the preferred ligand for the retinoid X receptor (RXR). The binding of these ligands to their respective receptors is essential for reproduction, growth, maintenance of skin and mucous membranes, and immunocompetence (*4*, *5*). The differences in transcriptional activation mediated through RXR and RAR binding have stimulated further interest in the absorption and metabolism of BC isomers.

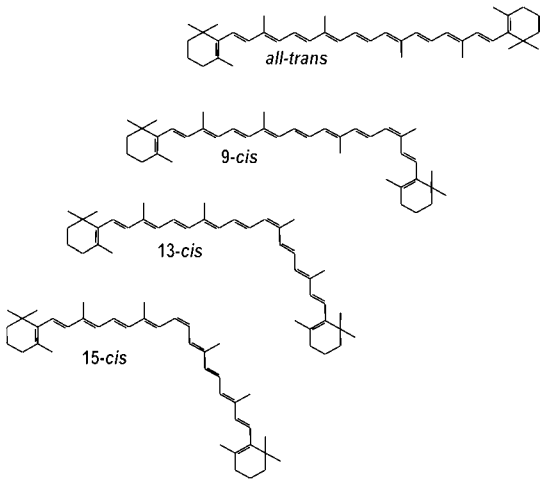

Figure 1. Structure of all-*trans*-BC and predominant cis isomers.

Although it is possible to consume significant amounts of *cis*-BC in processed foods, BC in human plasma and tissue samples is predominantly in the all-trans configuration (*6*). Insights about the basis for the different ratios of BC isomers

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in foods and body fluids and tissues remain limited. The absorption of dietary carotenoids requires that the plant pigments are transferred from the food matrix to micelles during digestion, delivered to the apical surface of absorptive epithelial cells, and packaged within chylomicrons for secretion into lymph (*7*, *8*). Stahl et al. (*9*) reported preferential transfer of ingested all-*trans*-BC as compared to 9-*cis*-BC to plasma chylomicron and VLDL fractions of human plasma. Deming et al. (*10*) reported that all-*trans*-BC was more bioavailable than 9- or 13-*cis*-BC in gerbils and suggested that this was due to the preferential uptake and accumulation of the all-trans isomer. Similarly, Caco-2 human intestinal cells preferentially accumulated all-*trans*-BC as compared to 9-*cis*-BC from Tween micelles (*11*). Although it has been generally assumed that carotenoids diffuse across the cell surface, recent reports suggest that uptake of all-*trans*-BC and lutein is mediated in part by scavenger receptor class B type 1 protein in Caco-2 cells and mice (*12*−*14*). Collectively, these data suggest that differences in transfer of *cis*- and all-*trans*-BC into lipid micelles from food matrix during digestion and their uptake by and secretion from intestinal mucosal cells may affect the profile of these carotenoids in fluids and tissues.

We have previously used in vitro digestion and the Caco-2 human intestinal cell model for systematic investigation of physical, chemical, and biological factors affecting partitioning of carotenoids and chlorophylls from various food matrices into mixed micelles and the subsequent uptake of the micellarized pigments by intestinal cells (*15*−*17*). The in vitro digestion model assesses carotenoid partitioning into nonuniform or mixed micelles that vary in size and composition as opposed to the more uniform, nonphysiological properties of synthetic micelles. Here, we used these models to study the influence of isomeric structure of BC on digestive stability, efficiency of micellarization, cell uptake, and intracellular stability. Water-soluble BC beadlets (WSB) and *Dunaliella salina* (DS) were the sources of the BC isomers.

MATERIALS AND METHODS

Materials. BC WSBs (10% BC wet wt) and Betatene (7.5% BC wet wt), a proprietary algal blend of DS, were gifts from Dr. Cindy Schweitzer, Cognis Corp. (Cincinnati, OH). Applesauce and corn oil were purchased at a local grocery store. Synthetic crystalline BC (95% all-trans), sodium bicarbonate, pepsin (porcine), pancreatin (porcine), bile extract (porcine), pancreatic lipase (porcine), butylated hydroxytoluene (BHT), sodium taurocholate, and protease (*Streptomyces griseus*; activity = 5.6 units/mg) were purchased from Sigma Chemical Co. (St. Louis, MO). Dulbecco's minimum essential medium (DMEM), fetal bovine serum, L-glutamine, amphotericin, gentamycin, and nonessential amino acids also were from Sigma Chemical Co. High-performance liquid chromatography (HPLC) reagent grade solvents were purchased from Fisher Scientific Co. (Norcross, GA). Ammonium acetate (Fluka, Ronkonkoma, NY) was dissolved in water (1 M solution) and adjusted to pH 4.6 using glacial acetic acid.

Preparation of Test Meals. The components of the test meal were applesauce (82−85% of total wet wt), corn oil (∼7.5% wet wt), and either synthetic BC WSBs or Betatene (DS) (8−10% wet wt). These materials (10 g) were homogenized (Tekmar Tissumizer, Cincinnati, OH) in 160 mL of saline (120 mmol/L NaCl containing 150 μmol/L BHT) for 90 s to produce a homogeneous meal. Final concentrations of BC in homogenized meals containing WSB and DS were 9.66 ± 0.05 and 6.28 ± 0.02 mg BC/g, respectively. Several aliquots (2 mL) of the homogenized test meal were transferred to 15 mL screw-top polypropylene tubes (Sarastedt, Newton, NC), blanketed with nitrogen, and stored at −80 °C until analysis for the starting quantities of BC isomers.

In Vitro Digestion. The procedure described by Garrett et al. (*15*) was used to simulate the gastric and small intestinal phases of digestion with minor modifications. Alterations included the addition of pancreatic lipase (10 mg in 0.1 mol/L sodium bicarbonate) to the small intestinal phase to ensure complete digestion of the oil and adjustment of the pH to 7.0 ± 0.1 with 1 mol/L NaOH for the small intestinal phase of digestion. After completion of the gastric and small intestinal phases, aliquots (2 mL) were transferred to 15 mL tubes (Sarstedt), blanketed with nitrogen, and stored at −80 °C until analysis for determination of BC stability during simulated digestion. Digestive stability represents the percentage of BC isomers in the test meal recovered in the digesta.

Samples (6 mL) of digestate were centrifuged (50.3 Ti rotor) at 167000*g* at 4 °C for 30 min (Beckman L7-65 Ultracentrifuge, Beckman Instruments, Palo Alto, CA) using heat-sealable ultracentrifuge tubes (Beckman Life Sciences, Brea, CA) to separate aqueous and residual particulate fractions. The micellar fraction was prepared by filtration (0.22 μm pore; Gelman Sciences, Ann Arbor, MI) of the aqueous fraction to remove microcrystalline particles of BC. Micellar fractions were either placed on ice for cellular uptake experiments (see below) or blanketed with nitrogen and stored at −80 °C until analyses to assess micellarization of BC isomers. Efficiency of micellarization is the percentage of BC isomers in the test meal that partitioned into micelles during simulated digestion. All manipulations were performed under dim or yellow light to protect the integrity of the BC isomers.

Uptake of Micellar Carotenoids by Intestinal Cells. Conditions for the growth and differentiation of Caco-2 cells (HTB-37, American Type Culture Collection, Rockville, MD) have been described previously (*18*). Cultures of Caco-2 were used between passages 23 and 36, and experiments were performed when monolayers were 10−13 days postconfluent. Experiments for examining uptake and retention of BC from micelles generated during digestion of WSB and DS were performed on the same day with replicate cultures to facilitate direct comparison of results. Monolayers were washed 1× with basal DMEM before adding 2 mL of the test medium containing 1.5 mL of DMEM and 0.5 mL of micellar fraction to cultures. The total BC contents in test media were approximately 5 and 11 mM for DS and WSB, respectively. This level of BC is below the level previously reported to saturate BC uptake in highly differentiated cultures of Caco-2 cultures (*31*). Cultures were incubated at 37 °C in a humidified atmosphere of 95% air/5% CO_2 (v/v) for 4 h. Then, medium was removed by aspiration and monolayers for one-half of the cultures were washed once with phosphate-buffered solution (PBS) containing 5 mmol/L sodium taurocholate at 0 °C to remove micelles and carotenoids adhering to the surface of monolayer. Replicate cultures were washed 1× with PBS containing 5 mmol/L sodium taurocholate at 37 °C before adding fresh DMEM (2 mL) without carotenoids. Cultures were returned to the incubator for an additional 14 h in order to investigate possible metabolism, isomerization, and efflux of the accumulated carotenoids under standard conditions. Cells were collected by scraping into 1.4 mL of ice-cold PBS and pelleted by centrifugation at ∼875*g* for 5 min. The supernatant was aspirated before cell pellets were overlaid with nitrogen gas and stored at −80 °C until analysis.

Stability of Micellar BC Isomers in Tissue Culture Environment. Filter-sterilized aqueous fractions were diluted 1:4 in DMEM, and aliquots (2.5 mL) were transferred into six well plastic culture dishes without cells. Dishes were incubated at 37 °C in a humidified atmosphere of 95% air/5% CO_2 (v/v). Samples were collected from 0 to 18 h, overlaid with nitrogen, and stored at −80 °C until analysis.

Extraction and Analysis of BC Isomers. Frozen DMEM containing aqueous fractions and cells were thawed. Cell samples were incubated in a 37 °C shaking water bath with 200 μL of protease (100 mg/10 mL PBS) (Sigma) for 30 min. Samples were then vortexed for 45 s with 500 μL of sodium dodecyl sulfate (SDS)−ethanol−BHT (1.0 mL of a 20% SDS−water solution dissolved in 19.0 mL of ethanol). Each sample was then extracted 3× using 500 μL of petroleum ether:acetone (3:1) containing 0.1% BHT (w/v). Samples were centrifuged for 1 min at 10000*g* between each extraction to facilitate phase separation. Pooled extracts were then dried under a stream of nitrogen before being redissolved in 400 μL of methyl *tert*-butyl ether (MTBE):methanol (50:50). Aliquots (100 μL) were analyzed by reverse-phase HPLC.

HPLC analysis of BC isomers was achieved as described by Ferruzzi et al. (*19*) with modifications. A Hewlett Packard model 1050 solvent delivery system and autosampler (Santa Clara, CA) with an ESA model

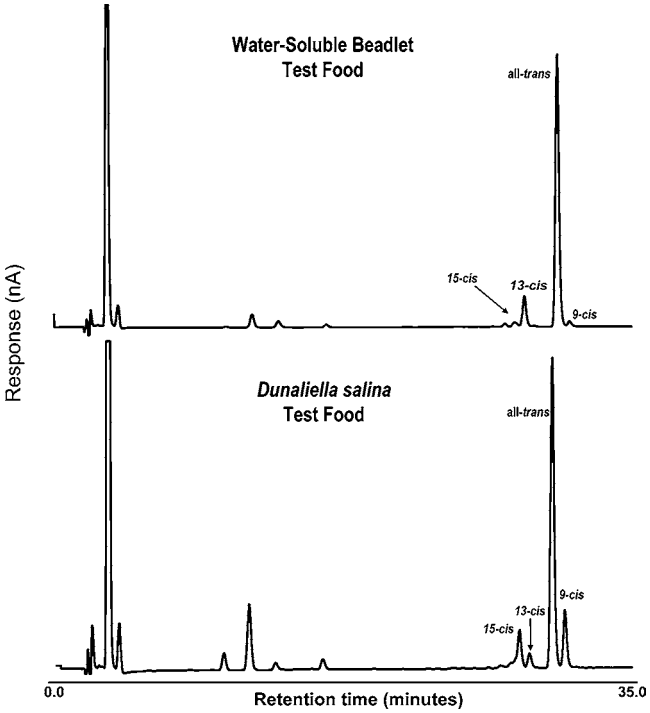

Figure 2. Representative chromatograms of carotenoids in test meals containing either BC WSBs or DS. All-*trans*-, 9-*cis*-, 13-*cis*-, and 15-*cis*-BC were separated by RP C30 HPLC with coulometric electrochemical array detection. The dominant response channel 5 (440 mV) is depicted at 500 nA full scale.

5600 Coularray electrochemical detector (Chelmsford, MA) equipped with eight channels in series was used for analysis. Potential settings from channels 1 to 8 were set at 200−620 mV in 60 mV increments. An analytical-scale 4.6 mm i.d. × 150 mm, 5 μm polymeric C_{30} column (YMC, Wilmington NC) with a guard column packed with C_{18} stationary phase was used.

Data Analysis. Data are expressed as means ± standard errors of the mean (SEM). The data were analyzed for differences between means using one-way analysis of variance (ANOVA). The Tukey−Kramer honestly significant difference test was used as a posthoc comparison of statistical significance. All statistical analyses were performed using the JMP statistical software program (SAS Institute, Cary, NC). Statistical significance was set at an α level of $P < 0.05$.

RESULTS AND DISCUSSION

The present study was designed to assess the digestive stability, efficiency of micellarization, and bioaccessibility of BC isomers from a simple test meal containing commercial BC supplements that contained both cis and all-trans isomers of the carotenoid. Applesauce was selected as the bulking component of BC-enriched test meals because it does not contain measurable levels of carotenoids and facilitates the incorporation of oil and BC sources into a homogeneous mixture. Corn oil (7.5% wet wt) was added to the test foods because it contributes negligible amounts of BC, provides a lipid source for the formation of mixed micelles, and is relatively resistant to oxidative degradation. BC WSBs and DS were selected as the sources of BC. Representative HPLC chromatograms of extracts of the two test meals are presented in **Figure 2** and demonstrate resolution of all-trans, 9-, 13-, and 15-cis isomers of BC. The meals with WSB and DS contained 37.7 ± 0.8 and 27.2 ± 1.8 μmol BC per simulated digestion reaction (50 mL total volume), respectively (**Table 1**). Contributions of individual BC isomers to total BC in WSB were 88% all-trans, 2% 9-cis, 8% 13-cis, and 2% 15-cis. DS generally contains similar levels of 9-*cis*- and all-*trans*-BC. However, the profile of BC isomers in the food with DS was 69% all-trans, 15% 9-cis, 7% 13-cis, and 10% 15-cis. The manufacturer noted that the growing conditions and time of harvest can affect the isomeric profile of the DS product (personal communication).

Table 1. Composition of BC Isomers in Test Foods[a,b]

	μmol/simulated digestion	
	WSBs[c]	DS[d]
total BC	37.7 ± 0.8	27.2 ± 1.8
	isomer	
all-trans	33.1 ± 0.8 (88%)[e]	18.7 ± 1.3 (69%)
9-cis	0.7 ± 0.1 (2%)	4.0 ± 0.1 (15%)
13-cis	3.2 ± 0.3 (8%)	1.8 ± 0.1 (7%)
15-cis	0.7 ± 0.0 (2%)	2.7 ± 0.2 (10%)

[a] Data are means ± SEM; $n = 6$. [b] Test food composed of applesauce (82–85% wet wt), corn oil (~7.5% wet wt), and BC source (8–10% wet wt). Tubes contained 2 g of food for each digestion reaction. [c] Food contains ~10 mg of BC/g food from WSB. [d] Food contains ~6 mg of BC/g food from DS. [e] The number in parentheses indicates percent of total BC represented by each isomer.

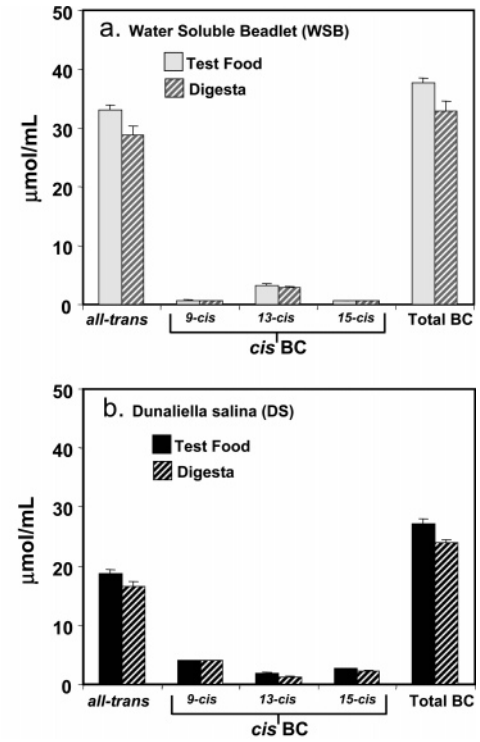

Figure 3. BC isomers are stable during simulated digestion of test foods containing WSB (**A**) and DS (**B**). The profiles of BC isomers in test foods (solid bars) and digesta (striped bars) were determined from six independent samples. There was no significant difference ($P > 0.05$) between the total concentrations of total BC or the individual isomers of BC after either test food was subjected to the gastric and small intestinal phases of digestion.

The qualitative and quantitative profiles of BC isomers after simulated gastric and small intestinal digestion of test foods containing WSB and DS were similar ($P > 0.05$) to those in the predigested test food (**Figure 3**). This demonstrates that BC isomers in the WSB and DS matrices mixed with the applesauce and oil were stable during simulated gastric and small intestinal phases of digestion. Likewise, BC isomers were stable during transit through the stomach and small intestine of human subjects, since the ratios of all-*trans*- and 9-*cis*-BC isolated from

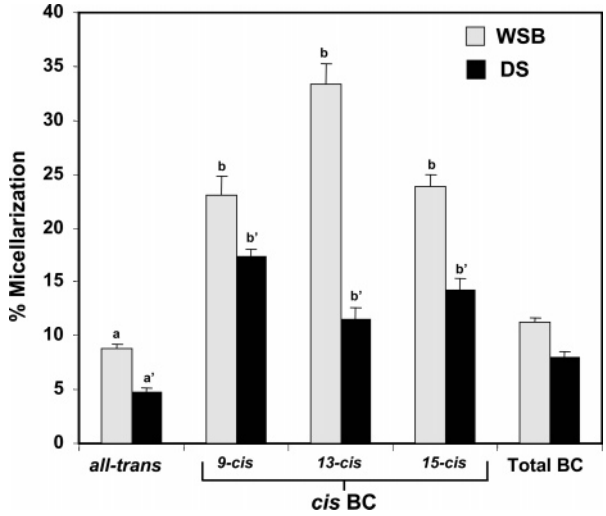

Figure 4. Cis isomers of BC are micellarized more efficiently than all-*trans*-BC during simulated digestion. The relative amount of BC isomers transferred from the test meal to the filtered aqueous fraction during digestion represents the efficiency of micellarization. Data are means ± SEM from six independent digestions. Efficiency of micellarization of total BC during digestion of food with WSB (open squares) was significantly (P <0.05) greater than for digested food containing DS (filled squares). Micellarization of all-*trans*-BC during digestion of samples containing either WSB or DS was significantly (P < 0.05) less than that of the cis isomers of BC as indicated by the different letters above the error bars. Micellarization efficiencies for 9-cis, 13-cis, and 15-cis isomers from digested test foods with either WSB or DS were not significantly different (P > 0.05).

DS were similar in the material ingested and the ileal effluent from human ileostomy subjects (*20*). In contrast, Deming et al. (*10*) reported partial isomerization of 9- and 13-*cis*-BC to all-*trans*-BC in the stomach and small intestine of gerbils given oral doses of each isomer solubilized in oil.

The efficiency of micellarization of total BC significantly (p < 0.05) differed for meals containing WSB and DS (11.2 ± 0.5 vs 8.0 ± 0.6%, respectively). However, micellarization for all BC cis isomers was more efficient (p < 0.05) than all-*trans*-BC for digested meals containing either WSB or DS (**Figure 4**). Cis isomers were incorporated into mixed micelles 2−3-fold more efficiently than all-*trans*-BC during simulated digestion, whereas the efficiencies of micellarization of the three cis isomers of BC were similar to one another (p > 0.05). Micellarization of *cis*-BC isomers during digestion of test meals ranged from 12 to 24% of that in the digesta. Despite the greater efficiency of micellarization for *cis*-BC, the quantity of all-*trans*-BC (2.9 ± 0.2 μmol per reaction; 69 ± 1% of total in micelles) exceeded (p < 0.05) that of the *cis*-BC isomers (1.4 ± 0.0 μmol per reaction; 59 ± 0.5%) for the digested meal with WSB as a result of the higher content of all-*trans*-BC in the test meal. For test meals containing DS, the more efficient micellarization of *cis*-BC isomer resulted in a greater (p < 0.05) quantity of *cis*-BC (1.3 ± 0.0 μmol per reaction) as compared to all-trans isomer (0.9 ± 0.0 μmol per reaction) in the micellar fraction.

The matrix in which BC is located in DS differs from that of the synthetic WSB and may affect BC partitioning during digestion. Ben-Amotz et al. (*21*) identified BC-containing globules in the interthylakoid spaces of the chloroplast in *Dunaliella bardawil*, a species similar to DS. BC represented more than one-half of the contents in the globule with the remainder primarily composed of neutral lipid. The localization of BC in chloroplasts likely contributed to the decreased

Table 2. Profile of BC in Caco-2 Cells Exposed to Micelles Generated during Digestion of Test Meal Containing BC WSBs

	pmol/well	pmol/mg protein	
		cellular BC content	
	micellarized BC in DMEM[a]	4 h (load)	18 h (load + retention)
total BC	22139 ± 1077[b]	1029 ± 66	916 ± 79
	BC isomers		
all-trans	15437 ± 1262 (70%)[c]	847 ± 55 (82%)*[d]	769 ± 62 (84%)
9-cis	884 ± 81 (4%)	43 ± 4 (4%)	36 ± 4 (4%)
13-cis	4988 ± 173 (23%)	126 ± 8 (12%)*	83 ± 7 (9%)**[e]
15-cis	831 ± 153 (4%)	13 ± 1 (1%)	27 ± 7 (3%)**

[a] BC isomers micellarized during in vitro digestion of food containing BC WSBs were diluted 1:4 in DMEM and added to cultures of differentiated Caco-2 HTB-37 cells. After 4 h, the medium was removed and cells were either collected for quantification or incubated with BC-free medium for an additional 14 h as described in the Materials and Methods. Carotenoids were extracted from the micellar fraction and Caco-2 cells and analyzed by HPLC. [b] Values are means ± SEM for n = 6. [c] The number in parentheses indicates the percent of total BC represented by each isomer. [d] Asterisk (*) as superscript indicates that the percent of total BC represented by each isomer in the diluted micellar fraction and after 4 h differs significantly (P < 0.05). [e] A double asterisk (**) indicates a significant difference (P < 0.05) in the percent of total BC represented by each isomer in cells after loading (4 h) and overnight incubation in carotenoid-free medium (18 h).

micellarization of BC during the digestion of food containing DS as compared to WSB. Indeed, it is well-established that the bioavailability of carotenoids from oil-based supplements is markedly greater than from plant foods (*22*). It is generally accepted that carotenoids must be solubilized in bile salt micelles for transfer across the unstirred water layer adjacent to the apical surface of the enterocyte. 9-*cis*-BC is readily solubilized in lipid and is more soluble in hydrophobic solvents than all-*trans*-BC (*23*). In fact, all-*trans*-BC is practically insoluble in oil and often precipitates as red crystals, while 9-*cis*-BC resists crystallization and can exist as an oil at high concentrations. These physiochemical properties of 9-*cis*-BC may allow it to serve as a solvent for the all-trans isomer explaining observed increases in absorption of total BC in the presence of the 9-cis isomer (*23*, *24*).

The micellar fractions containing BC isomers from digested foods supplemented with WSB and DS test meals were diluted (1:4) with serum-free DMEM and added to cultures of differentiated Caco-2 cells to examine cellular uptake. Cellular BC content (pmol per mg/protein) represented approximately 5 and 9% that in medium after monolayers were incubated for 4 h in medium containing micelles generated during digestion of meals with WSB and DS, respectively (**Tables 2** and **3**). The intracellular profile of BC isomers differed slightly from that of the micellarized fraction from digested WSB with a significant (P < 0.05) increase and (P < 0.05) decrease in the relative amounts of all-trans and 13-cis isomers of BC, respectively, in cells (**Table 2**). In contrast, there were no significant (P > 0.05) differences in the profile of BC isomers in Caco-2 cells and medium containing micelles generated by digestion of food with the DS supplement (**Table 3**). Following overnight (14 h) incubation of monolayers preloaded with BC in carotenoid-free medium, the cellular content of BC declined by 12 ± 3 and 32 ± 9% for WSB and DS treatment groups, respectively (**Tables 2** and **3**). Significant (P < 0.05) changes in the relative amounts of intracellular BC isomers during overnight incubation were limited to a decline and an increase in 15- and 13-*cis*-BC, respectively, in cells previously exposed to micelles generated during digestion of meal containing WSB.

Table 3. Profile of BC in HTB-37 Cells Following Cellular Uptake of Digested Food Fortified with DS

	pmol/well	pmol/mg protein	
		cell BC	
	micellarized BC in DMEM[a]	4 h (load)	18 h (load + retention)
total BC	11384 ± 473[b]	978 ± 120	740 ± 171
	BC isomers		
all-trans	5258 ± 516 (46%)[c]	482 ± 62 (49%)[d]	403 ± 75 (58%)[e]
9-cis	3277 ± 238 (29%)	273 ± 31 (30%)	200 ± 55 (25%)
13-cis	993 ± 87 (9%)	89 ± 12 (9%)	67 ± 20 (8%)
15-cis	1856 ± 124 (16%)	118 ± 18 (12%)	71 ± 21 (9%)

[a] Carotenoid accumulation and retention by Caco-2 cells incubated with the micellar fraction generated during digestion of the meal containing DS were determined as described for **Table 2**. [b] Values are means ± SEM for $n = 6$. [c] The number in parentheses indicates percent of total BC represented by each isomer. [d] There were no significant differences ($P > 0.05$) in the percent of total BC represented by each isomer in the medium added to cultures and in cells after 4 h. [e] There were no significant differences ($P > 0.05$) in the percent of total BC represented by each isomer in cells after loading (4 h) and overnight incubation in carotenoid-free medium (18 h).

Our observation that uptake of micellarized all trans and cis isomers of BC by Caco-2 cells was proportional to their amounts in medium is in line with results reported by other investigators. Deming et al. (*10*) observed preferential uptake of *cis*-BC from the small intestinal lumen after feeding gerbils oil enriched with all-*trans*- and either 9- or 13-*cis*-BC. Moore et al. (*25*) also observed similar uptake kinetics of all-*trans* and 9-*cis*-BC by intestinal brush-border membrane vesicles. Perfusion of ferret small intestine with 9-*cis*-BC resulted in a 10-fold greater quantity of the cis isomer in the mucosa and a similar degree of transport of the isomer into mesenteric lymph as compared with the accumulation and transport of all-*trans*-BC (*26*). In contrast, During et al. (*11*) recently reported that the uptake of all-*trans*-BC by Caco-2 cells from Tween 40 micelles was significantly greater than that of preparations containing 9-*cis*- and 13-*cis*-BC. While the basis for the discrepancy for the uptake of BC isomers by Caco-2 cells reported here and those of During and colleagues is not known, it is possible that the use of mixed micelles generated during simulated digestion and the nonionic detergent as delivery vehicles may have affected uptake. Artificial micelles prepared with Tween 20 and hexadecyltrimethylammonium bromide reportedly facilitate absorption of retinol but not BC, suggesting that bile salts may play a role in BC absorption beyond the simple solubilization of the carotenoid in micelles (*27*). Also, the concentration of micellarized BC isomers in the present study was approximately 1 order of magnitude higher than that used by During et al (*11*), i.e., approximately 5−10 vs 1 μM, respectively, and this may have minimized isomer specific differences mediated by a facilitated uptake process.

13-*cis*-BC, the main cis isomer in plasma, does not exceed 7% of the total amount of plasma BC, and 15-*cis*-BC is not detected in plasma (*28*). Caco-2 cells accumulated both of these isomers when incubated with micelles generated during simulated digestion. You et al. (*6*) reported that the great majority of BC and retinol appearing in plasma were (^{13}C) all-trans isomers after human subjects were administered (^{13}C) 9-*cis*-BC. This suggests that potential for preabsorptive cis to trans isomerization in vivo. Our in vitro observations suggest that such isomerization is limited during digestion, following partitioning of BC into micelles, during transfer from micelles to the absorptive cells, and within the cells when cultures are incubated in the fasting-like state, i.e., without extracellular lipid required for stimulating chylomicron biosynthesis (*11*, *29*). However, conditions favorable to cis−trans isomerization reactions may exist during processes required for the incorporation of intracellular BC into chylomicrons or after secretion into lymph. Isomer specific differences in the intracellular metabolism and transport, degree of incorporation into chylomicrons, and rate of postabsorptive transfer to tissues for BC require systematic investigation.

BC isomers solubilized in bile salt micelles were stable for as long as 18 h when diluted in basal medium and incubated under standard culture conditions (data not shown). Similar observations were reported previously for micellarized lutein and lycopene in cell culture environment (*29*, *30*). It is important to note that a low concentration of BHT was present during simulated digestion (see Methods), and both sources of BC also contained tocopherols. These antioxidants protect carotenoids added to cell culture medium during storage of samples (−20 ° C in the dark in an atmosphere of nitrogen) (*24*, *30*).

In conclusion, the in vitro digestion method coupled with the Caco-2 human intestinal cell line provided a physiologically relevant model for examining the digestion, micellarization, and intestinal cell uptake of BC isomers. BC isomers in WSB and DS were stable during the gastric and small intestinal phases of digestion when ingested with foods. Cis isomers of BC were micellarized more efficiently than all-trans isomers, and micellarized isomers were stable in the cell culture environment. The similar profile of carotenoids in micelles and Caco-2 human intestinal cells suggests that uptake was not isomer specific at the concentrations used in this study. Further investigations are needed to better characterize the molecular processes of intestinal cell uptake, metabolism, and transport of BC isomers that may contribute to observed differences between carotenoid isomeric profiles in food and tissues.

ABBREVIATIONS USED

BC, β-carotene; WSB, water soluble beadlet; DS, *Dunaliella salina*; HPLC, high-performance liquid chromatography; RA, retinoic acid.

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