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Accumulation and retention of micellar β-carotene and lutein by Caco-2 human intestinal cells

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Despite the interest in the diverse roles of dietary carotenoids in human health, little is known about the transfer of these plant pigments from foods to micelles during digestion and their subsequent transfer across the intestinal epithelium. We conducted this study to characterize the intestinal uptake of micellarized carotenoids using monolayers of differentiated Caco-2 human intestinal cells. Crystalline β-carotene (BC) and lutein (LUT), solubilized in mixed micelles for delivery to cells, were stable in a tissue culture environment for 20 hours. Cellular accumulation of micellar BC and LUT was proportional to the media content of carotenoids at ≤2 μmol/L and the length of exposure. Cellular accumulation of BC routinely exceeded LUT and was due in part to the enhanced efflux or possible metabolism of LUT. Cellular BC content increased in a curvilinear manner when cultures were incubated in micellar medium containing 2 to 27 μmol/L BC prepared from water miscible beadlets; cellular BC content was maximum when medium BC was ≥18 μmol/L. There was no indication that high levels of BC in medium or within cells adversely affected micellar LUT accumulation. These data support the use of the Caco-2 human cell line as a model for studying the intestinal uptake, absorption, and possible interactions of dietary carotenoids. (J. Nutr. Biochem. 10:573–581, 1999)

Keywords: β-carotene; lutein; micelles; carotenoid interactions; Caco-2 cells

Introduction

Epidemiologic studies have revealed that consumption of carotenoid-rich fruits and vegetables is correlated with a reduction in the incidence of certain types of cancer and cardiovascular disease.[1,2] Carotenoids have been proposed to be beneficial for health because they exhibit antioxidant[3] and immunomodulatory[4,5] activities and inhibit neoplastic transformation,[6] and because many are metabolized to retinoids.[7,8]

The majority of studies related to carotenoid absorption have been limited to β-carotene (BC), primarily because of its pro-vitamin A activity, relatively high abundance in human plasma, and Food and Drug Administration approval for ingestion by humans. The absorption and metabolism of other carotenoids that are common in foods have received increased attention. For example, the oxycarotenoid lutein (LUT) is of interest because of its potential role in the prevention of age-related macular degeneration[9] and its inhibition of methylcholantrene-induced neoplastic foci in cell cultures.[10] Carotenoid absorption by humans has been assessed primarily by monitoring its rise and decline in the plasma following acute or chronic administration.[11] Although this approach has been informative, it does not quantify the amount of carotenoid that is absorbed. Use of animals for the study of carotenoid absorption has been limited because the efficiency of carotenoid absorption is species dependent. For example, rat small intestine converts virtually all BC to retinol[12]; in contrast, BC is only partially cleaved to retinoids and apo-carotenals in ferret and preruminant calf intestine, a situation that is similar to that which occurs in humans.[13–15] The special needs of these animal models restrict their widespread use in carotenoid research. Others have addressed the intestinal carotenoid transport of

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BC using perfused sections of rat[16] and ferret[13] intestine, a rat intestinal cell line,[17] and brush border membrane vesicles from rat small intestine.[18] However, our current understanding of the mechanisms of digestion, absorption, and metabolism of dietary carotenoids remains limited. Parker[19] has recommended the development of alternate in vitro models to study such processes.

The Caco-2 human intestinal cell line appears to represent a potentially useful model for studying the metabolism and transport of dietary carotenoids by intestinal absorptive cells. Caco-2 cultures spontaneously differentiate at confluency into cells that exhibit phenotypic properties that are similar to those of mature enterocytes, including a highly developed brush border, tight junctions that minimize paracellular transport, and basolateral secretion of chylomicrons and very low density lipoproteins.[20,21] This cell line has been used to examine selective characteristics of the intestinal absorption of the lipophilic micronutrients vitamin E[22] and retinol.[23,24] Moreover, it has been reported that some passages of Caco-2 cells cleaved BC to retinol and synthesized retinyl esters.[25] The primary objective of the present study was to characterize the uptake and accumulation of micellar BC and LUT (i.e., carotenoids with and without pro-vitamin A activity, respectively) by differentiated cultures of Caco-2 cells. The possible interaction between BC and LUT also was examined when both carotenoids were simultaneously added to cultures and after cellular preloading with BC.

Material and methods

Supplies

Synthetic crystalline BC (95% all-trans), LUT (95–98% all-trans from marigolds), and water miscible BC beadlets (10% BC by weight; Hoffmann-La Roche, Inc., Nutley, NJ USA) were kindly provided by Dr. J. Cecil Smith, Jr. (U.S. Department of Agriculture Human Nutrition Research Center, Beltsville, MD USA). The purity of crystalline BC and LUT exceeded 97% as determined by high performance liquid chromatography (HPLC). Oleic acid, 2-mono-acyl-glycerol, phosphatidylcholine, sodium taurocholate, Dulbecco's minimum essential medium (DMEM), fetal calf serum (FCS), L-glutamine, amphotericin B, gentamicin, nonessential amino acids, sodium bicarbonate, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), and Hank's balanced salt solution (HBSS) were purchased from Sigma Chemical Co. (St. Louis, MO USA). All other reagents and materials were purchased from Fisher Scientific Co. (Norcross, GA USA).

Cell culture

Caco-2 cells (HTB-37) were obtained from American Type Culture Collection (ATCC, Rockville, MD USA) at passage number 19 and stock cultures were maintained in complete DMEM and handled as previously described.[26] Complete DMEM was prepared by supplementing basal DMEM containing 4.5 g/L glucose (D7777) with the following: 10% heat-inactivated FCS; nonessential amino acids (10 mL/L medium; M7145); L-glutamine (2 mmol/L); amphotericin B (0.5 mg/L); gentamicin (50 mg/L); HEPES buffer (15 mmol/L); and sodium bicarbonate (44 mmol/L). For experiments, cells were seeded in 12-well multiwell plastic dishes (Beckton Dickinson Labware, Franklin Lakes, NJ USA) at a density of 1.5×10^5 cells/well and incubated in a humidified atmosphere of 95% air/5% carbon dioxide (CO_2) at 37°C. Spent medium was replaced every 2 days with fresh complete medium and cultures were used for experiments between 11 and 14 days postconfluency, because cultures of Caco-2 cells exhibit maximum differentiation at this age as assessed by the activities of alkaline phosphatase and sucrase.[27]

Micellar preparations of crystalline carotenoids

BC and LUT were delivered to Caco-2 cells in mixed micelles that were prepared as described by Canfield et al.[28] and modified by Martin et al.[29] The photodecomposition and oxidation of the carotenoids was minimized by performing all manipulations under subdued yellow light and storing all solutions under a blanket of argon. Stock solutions of 2-mono-acylglycerol, oleic acid, and BC were prepared in hexane, LUT in absolute ethanol, and phosphatidylcholine in chloroform:methanol (3v:1v). Stock solutions of carotenoids (1.5 mmol/L) also contained 2.2 mmol/L butylated hydroxytoluene (BHT). Appropriate volumes of each stock solution of the following compounds were transferred to glass bottles to give the following final concentrations after the addition of serum free and phenol red free DMEM containing 5 mmol/L sodium taurocholate: 300 μmol/L 2-mono-acylglycerol; 100 μmol/L oleic acid; 50 μmol/L phosphatidylcholine; 15 μmol/L carotenoid; and 22 μmol/L BHT. Micellar medium was prepared by sonication in a water bath (Sonic Systems Inc., Newton, PA USA) for three cycles of 5 minutes each with water changed after each cycle to minimize the thermal degradation of the carotenoids. The medium was filtered (0.22 μm pores) to remove carotenoids that were not incorporated into micelles[10] and microbial contamination. The likelihood that the carotenoids present in the filtrate had been incorporated into micelles was supported by several observations. First, filtrates ($n = 12$) from preparations containing crystalline carotenoids without micellar components did not contain detectable levels of BC or LUT as determined by HPLC (<1 μmole). Second, incubation of filtrates ($n = 9$) of micellar medium at 0°C for 1 hour did not result in any colored precipitate and more than 98% of the carotenoids passed through a second filter (0.22 μm). The efficiency of transfer of crystalline BC and LUT into micelles was 7 to 13% ($n = 12$) and 7 to 20% ($n = 12$), respectively, which is similar to that reported previously.[28,29]

When necessary, the filtrate was diluted with medium containing mixed micelles without carotenoids to prepare test media with varying concentrations of BC and LUT and an identical amount of micelles in all wells.

Micellar preparation of BC from water miscible beadlets

Water miscible beadlets containing 10% BC were added to DMEM without phenol red and FCS to yield a maximum starting concentration of 150 μmol/L BC. Aliquots were transferred to glass bottles with or without micellar components and the mixture was sonicated and filter-sterilized (0.22 μm pores) as above. Postfiltration recovery of BC from preparations containing starting concentrations of 2, 6, 15, 37.5, 75, and 150 μmol/L BC was 40.9 ± 2.3% ($n = 9$ for each concentration), a level that is several-fold higher than that obtained with preparations containing crystalline BC (see above). However, the following observations revealed that almost one third of the BC from the beadlet material that was present in the filtrate had not been incorporated into micelles. First, 12.9 ± 0.5% ($n = 9$ for each concentration) of beadlet BC added to medium without micellar components passed through the filter. Second, incubation of filtered micellar medium prepared with beadlet BC for 1 hour at 0°C resulted in the formation of an orange precipitate. This preparation was re-filtered and the amount of BC present in the second filtrate was only 71.6 ± 5.5% of the starting concentration. Consequently, we

limited our use of the beadlet preparation of BC to a single experiment in which cells were exposed to higher levels of the carotenoid than could be achieved when starting with crystalline BC. Aliquots of filtered media prepared with beadlet BC also were diluted with micelle-containing medium without BC to maintain a constant amount of micelles in all test media.

Cellular uptake of micellar carotenoids

Experiments were performed using cultures of the HTB-37 line of Caco-2 cells between the 22nd and 45th passage. The potential cytotoxicity of micellar medium on cultures was evaluated in pilot studies. Gross morphologic appearance, the number of domes per microscopic field (an indicator of basolateral Na, K-ATPase activity),[20] and the protein content per well were similar in differentiated cultures incubated in medium with and without micelles for as long as 24 hours. This demonstrated that exposure to micelles did not adversely affect cellular integrity.

To initiate experiments, monolayers were washed twice with 1 mL HBSS before adding either 1 mL micelle-containing medium either without carotenoids or with BC or LUT to triplicate wells of the 12-well culture dish. To examine possible interactions between LUT and BC, cultures were incubated in micellar medium containing either both carotenoids or LUT only when cells had been preloaded with BC (see Results). Cultures were incubated at 37°C. Carotenoid uptake was terminated at indicated times by removing spent medium and washing monolayers with HBSS containing 5 mmol/L sodium taurocholate at 22°C three times. Washed monolayers were harvested in 1 mL ice-cold phosphate buffered saline containing 10% (v/v) ethanol and 45 μmol/L BHT. Samples were stored at −70°C under a blanket of argon until analysis.

Retention of intracellular BC and LUT by Caco-2 cells

Several studies also examined the cellular retention of accumulated BC and LUT. Caco-2 cells were loaded with either BC or LUT by incubating differentiated cultures in medium containing micellar BC (1.96 ± 0.02 μM) or LUT (1.94 ± 0.04 μM), respectively, for 20 hours. The carotenoid-containing media were removed, and the monolayers were washed with HBSS containing 5 mmol/L sodium taurocholate to remove surface bound carotenoids. Starting carotenoid content of the cell monolayer was 298 ± 5 and 165 ± 3 pmoles/mg BC and LUT, respectively. Fresh complete medium was added to cultures and the quantities of BC and LUT in both cells and medium followed for 36 hours. Cells were collected as described above. In addition, spent media were centrifuged at 730 × g for 5 minutes at 4°C to remove any cellular debris, frozen in liquid nitrogen, and stored at −70°C prior to determining carotenoid content. The experiment was conducted three times with triplicate observations for each parameter tested ($n = 9$).

Analysis of carotenoids

Pilot studies showed that the levels of BC and LUT in freshly prepared cells and medium versus frozen cells and medium did not differ ($P > 0.01$) from replicate samples frozen at −70°C for as long as 5 days. Frozen samples were thawed and the cell homogenates sonicated on ice at a setting of 3 for 10 seconds (Sonic Dismembrator, Fisher Scientific Co.). Aliquots (500 μL) were extracted twice with hexane, dried under a stream of nitrogen, reconstituted in 500 μL of mobile phase (see below), and analyzed by reverse-phase HPLC (Hewlett-Packard, Model HP-1090, Avondale, PA USA) using an ultraviolet/visible dual beam detector with wavelength set at 450 nm. Carotenoids were separated[30] on a C-18 ODS Microsorb-MV 5 μm analytical column (25 cm × 0.46 cm diameter; Rainin, Woburn, MA USA) that was protected by a C-18 Adsorbosphere guard column with 5-μm particles (0.75 × 0.46 cm; Alltech, Deerfield, IL USA). The mobile phase was 70% (v/v) acetonitrile containing 13 mmol/L triethylamine, 20% (v/v) methylene chloride, and 10% (v/v) methanol containing 1.3 mmol/L ammonium acetate. Isocratic analyses were performed at a flow rate of 1.7 mL/min. The column temperature was maintained at 22°C by using a constant temperature circulator (Model 910, Fisher Scientific, McGraw Park, IL USA). Results were calculated from a multilevel calibration table derived from a series of external standards. Recovery (93.0 ± 0.9%) of carotenoids was monitored by addition of echinenone (0.45 μmol/L) to test samples. BC and LUT were not detected (<1 pmole) in cells that had been maintained under standard tissue culture conditions without exposure to micellar carotenoids. Aliquots of sonicated material were assayed for protein using the bicinchininoic acid assay (Pierce, Rockford, IL USA).

Statistical analysis of data

The data represent the mean ± SEM for either two or three experiments with triplicate observations in each experiment. Data for *Figures 1, 2, 4, and 5* were analyzed by analysis of covariance, *Figure 3* by analysis of variance, and *Figure 6* using Student's *t*-test. Tukey-Kramer Honestly Significant Difference test was used as a post-hoc comparison of statistical significance. Statistical analyses were performed using the JMP statistical software program (SAS Institute, Cary, NC USA). All statistical analyses were performed at a *P*-value of less than 0.01.

Results

Characterization of BC and LUT accumulation by Caco-2 cells

The stability of the preparations of micellar BC and LUT in a cell-free tissue culture environment (i.e., a humidified atmosphere of 95% air/5% CO_2) was evaluated prior to characterizing the accumulation of micellar BC and LUT by Caco-2 cells. Serum-free DMEM containing micelles, 22 μmol/L BHT, and either 1.83 ± 0.06 μmol/L BC or 1.86 ± 0.04 μmol/L LUT was added to 12-well dishes and incubated at 37°C. The percentages of the initial concentrations of carotenoids recovered from the medium after incubation for 24 hours were 99 ± 2 and 79 ± 2% ($n = 9$) for BC and LUT, respectively.

The uptake of BC and LUT by Caco-2 cells was examined initially by incubating cultures in DMEM containing micelles with less than 2 μmol/L of a single carotenoid for 20 hours. Cellular BC content was directly proportional to the concentration of BC in medium ($y = 96.2x - 1.12$) and reached a level of 176 ± 9.7 pmol/mg cell protein when the initial medium content was 1.9 μmol/L (*Figure 1*). The mean cellular content of BC represented 9.6 ± 0.3% of the amount of the carotenoid added to cultures at the beginning of the exposure period. The cellular level of LUT also increased proportionally as the concentration of LUT in micellar medium was elevated ($y = 47.9 \times 9.7$; *Figure 1*). However, the mean cellular level of LUT only represented 6.4 ± 0.7% of that in medium containing from 0.53 to 1.90 μmol/L. Cellular content of BC exceeded ($P < 0.01$) that of LUT when the concentration of carotenoid in medium exceeded 0.5 μmol/L (*Figure 1*).

The impact of the length of exposure of cultures to

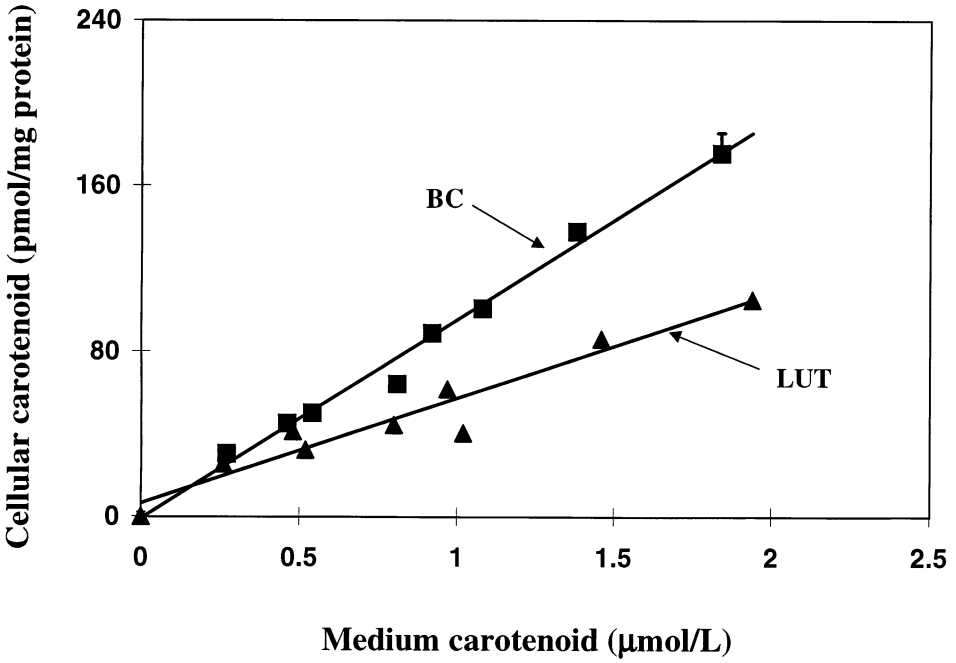

Figure 1 The effect of micellar concentration of carotenoids in medium on the uptake of β-carotene (BC) and lutein (LUT) by Caco-2 cells. Confluent cultures of Caco-2 were incubated in medium containing the indicated concentrations of micellar carotenoids for 20 hours. After removing spent medium, the monolayers were washed three times with Hank's balanced salt solution containing 5 mmol/L sodium taurocholate and collected in 1 mL phosphate buffered saline containing 10% ethanol (v/v) and 45 μmol/L butylated hydroxytoluene. Cells were sonicated and extracted in hexane to determine carotenoid content by high performance liquid chromatography as described in Materials and Methods. Each point represents the mean ± SEM of triplicate observations from three separate experiments. Cellular levels of BC were significantly ($P < 0.01$) greater than LUT when the concentration of the carotenoids in the medium exceeded 0.5 μmol/L.

micellar medium containing 1.9 μmol/L BC or LUT was examined. The evident feature of the results presented in *Figure 2* is that cellular content of BC was 2.0- to 2.7-fold higher ($P < 0.01$) than LUT after incubating cultures in media containing similar concentrations of either carotenoid for an identical period. In addition, cellular levels of the carotenoids increased more rapidly during the initial period of exposure than at later times. For example, the levels of BC and LUT after 4 hours represented 39% and 51%, respectively, of that present after 30 hours incubation. Finally, cells continued to accumulate BC from medium between 20 and 30 hours incubation ($P < 0.01$), whereas the quantity of LUT in cells did not change significantly ($P > 0.01$) during this period.

The relatively high concentrations of BC in medium achieved using the water miscible beadlet preparation provided the opportunity to further examine the relationship of extra- and intracellular BC concentrations than was possible when using crystalline BC. Data in *Figure 3* show that cellular accumulation of BC after an 8-hour incubation increased in a curvilinear manner when medium concentrations of BC ranged from 1.8 to 27 μmol/L. Cellular BC content appeared to be maximum when the concentration of BC in micellar media was 18 μmol/L or greater.

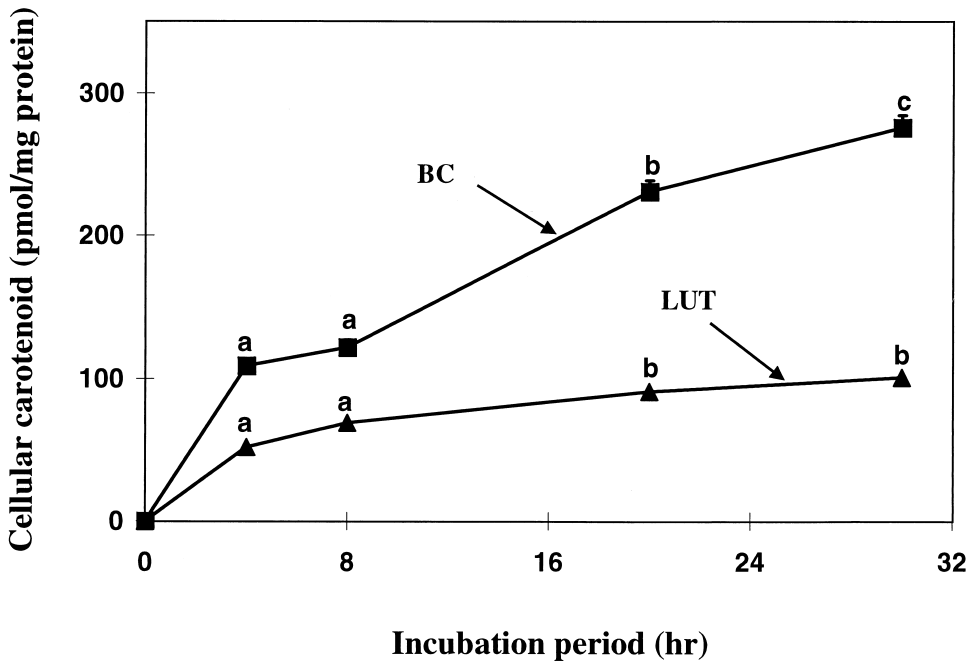

Figure 2 The influence of incubation period on cellular accumulation of β-carotene (BC) and lutein (LUT) from medium containing micelles. Confluent cultures of Caco-2 were incubated in medium containing micellar BC or LUT (1.9 μmol/L) for indicated times. After removing test media and washing the monolayers, cellular levels of carotenoids were measured as described in Materials and Methods. Each point represents the mean ± SEM of triplicate observations from three separate experiments. The cellular content of BC was significantly ($P < 0.01$) higher than that of LUT at each time of incubation. The presence of the same letter above the error bars within each line indicates that the cellular level of the carotenoid was similar ($P > 0.01$) at stated times of incubation.

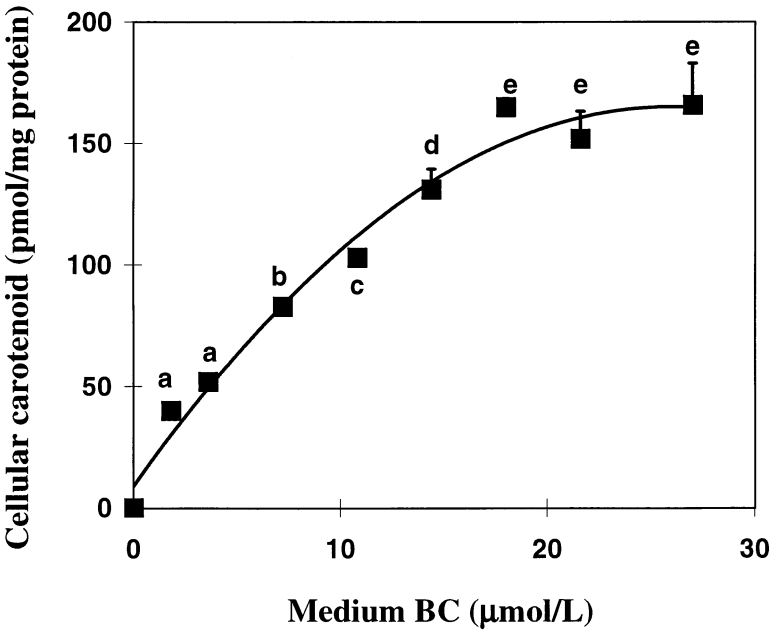

Figure 3 Cellular accumulation of micellar β-carotene (BC) prepared from water miscible beadlets. Differentiated cultures of Caco-2 cells were incubated for 8 hours in micellar medium containing 2 to 27 μmol/L BC. The presence of different letters above error bars indicates that the cellular accumulation of BC differed significantly ($P < 0.01$) at the media concentrations tested. Each point represents the mean ± SEM of triplicate observations from two experiments.

The greater accumulation of BC than LUT from micellar medium suggested that the uptake, metabolism, and/or efflux of these carotenoids might differ. The latter two possibilities were investigated. Cultures were pre-loaded with either BC or LUT by incubation in micellar medium (1.95 μmol/L carotenoid) for 24 hours. After removal of spent media and the addition of carotenoid-free medium, the quantities of BC and LUT in cells and medium were monitored for as long as 36 hours. Cells contained 298 ± 5.0 and 165 ± 3.0 pmoles/mg protein of BC and LUT, respectively, at 0 hours. Cells retained 94 ± 0.1% of the starting quantity of BC after incubation for 2 hours and 72 ± 0.9% by 36 hours (*Figure 4*). Medium levels of BC represented 3.5 ± 0.1% of that originally present in cells and increased steadily to 11.7 ± 0.5% by 36 hours. The decrease (16%) in the BC level in the culture (cells and medium) was not associated with an increase in retinol which was not detected (<0.07 μmol/L) in either cells or medium at any time. In contrast, cells retained 80 ± 0.4% and 50 ± 2% of the starting quantity of LUT after 2 and 36 hours, respectively. Medium LUT accounted for 9.5 ± 0.2% of that initially associated with cells after incubation for 2 hours and increased to 19.5 ± 0.5% after 36 hours. Thus, the recovery of starting concentration of LUT in cells and medium after 36 hours represented approximately 70% of that initially present in the cells at 0 hours.

Examination of potential BC and LUT interactions

Initially, equal volumes of micellar medium containing 2 μmol/L of either BC or LUT were combined before the addition of medium to Caco-2 cultures. After incubation in this medium for 8 hours, the cellular content of each carotenoid was similar ($P > 0.01$) to that of cultures that

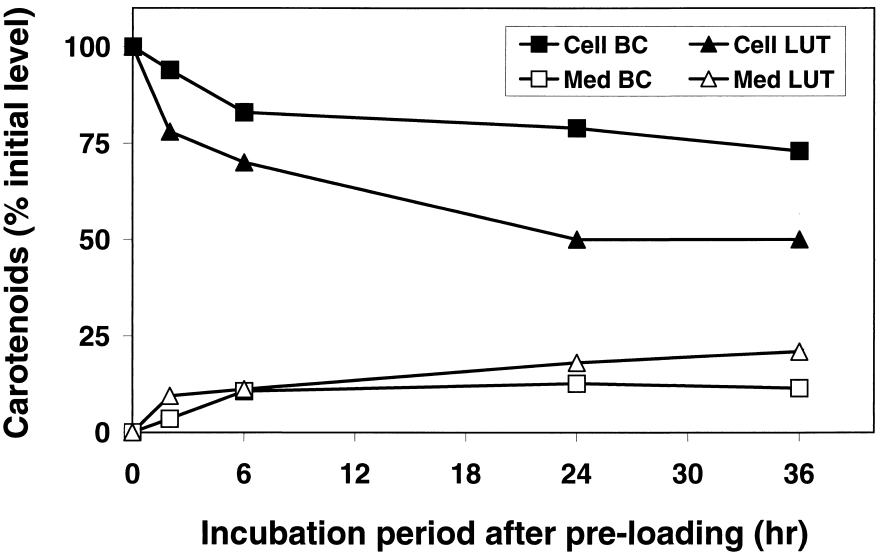

Figure 4 Retention of β-carotene (BC) and lutein (LUT) by Caco-2 cells. Caco-2 cultures were incubated in medium containing 1.95 μmol/L BC or LUT for 24 hours. Spent medium was removed, monolayers were incubated in fresh complete Dulbecco's minimum essential medium for indicated times, and the quantities of the carotenoids in cells and spent medium was determined. The percentage of LUT retained by cells was significantly ($P < 0.01$) lower than that of BC at all times examined. Medium LUT content was significantly greater than BC except after 2 hours when the concentrations of medium BC and LUT were similar. Each point represents the mean ± SEM of triplicate observations from three experiments.

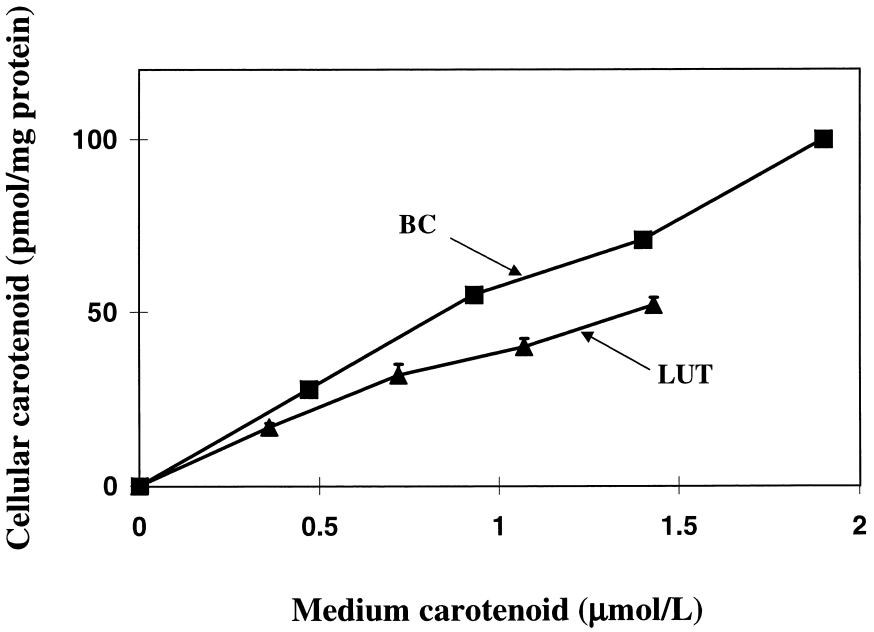

Figure 5 Uptake of β-carotene (BC) and lutein (LUT) by Caco-2 cells from micellar medium containing both carotenoids. Confluent cultures of Caco-2 cells were incubated in medium with indicated concentrations of micellar BC and LUT for 8 hours. Cellular levels of BC and LUT were measured as described in the legend for *Figure 1*. Each point represents the mean ± SEM of triplicate observations from three experiments. At similar medium concentrations, cellular accumulation of BC significantly ($P < 0.01$) exceeded that of LUT.

had been incubated in medium containing the same concentration of either BC or LUT for an identical period (data not shown). Next, micellar medium was prepared in the presence of both BC and LUT to increase the likelihood that both carotenoids were present within a micelle. The initial filtrate contained 1.9 μmol/L BC and 1.4 μmol/L LUT. Aliquots were diluted with medium containing micelles but no carotenoids to provide test media containing different concentrations of the carotenoids but a 4:3 molar ratio of BC to LUT (i.e., 0.36, 0.72, 1.07, and 1.43 μmol/L LUT and 0.47, 0.95, 1.43, and 1.9 μmol/L BC, respectively). As observed in the earlier studies with either BC or LUT (*Figure 1*), cellular levels of BC and LUT were proportional to the concentrations in medium and BC accumulation exceeded that of LUT (*Figure 5*).

The effect of relatively high levels of extra- or intracellular BC on uptake of micellar LUT was examined in light of previous results from human trials that revealed an interaction between these two carotenoids.[31,32] Beadlets containing water miscible BC were used as the carotenoid source in this experiment because it allowed us to markedly increase medium content of micellar BC (see Materials and Methods). In the first experiment, cultures were incubated in micellar medium containing 2.9 μmol/L LUT with or without 23.7 μmol/L BC for 8 hours. Cellular LUT content was 25% higher ($P < 0.01$) in cultures incubated in medium containing a high level of BC than in cultures treated with LUT alone (*Figure 6A*). Next, cultures were incubated initially in control medium (no BC) or medium containing 33 μmol/L BC for 24 hours to elevate cellular BC content. Control and BC-treated cultures contained less than 1 and 432 ± 25 pmoles BC/mg cell protein, respectively, after 24 hours. Fresh micellar medium containing 2.3 μmol/L LUT was added to all cultures after removing spent media. Cellular accumulation of micellar LUT was not altered ($P > 0.01$) by high levels of intracellular BC (*Figure 6B*).

Discussion

A series of studies was designed to characterize the intestinal uptake of BC and LUT using the Caco-2 human intestinal cell line. This necessitated the selection of a vehicle for delivery of carotenoids to the apical surface of differentiated cultures that would not compromise the morphologic or biochemical integrity of the cells. Mixed micelles were used to deliver carotenoids to Caco-2 cells because they facilitate the in vivo transfer of lipophiles across the unstirred water layer to the brush border surface of absorptive cells.[12,14] Moreover, we previously reported that micelles were an effective vehicle for delivering both BC and LUT to HepG2 human liver cells.[29] BHT also was incorporated in micellar preparations to stabilize the carotenoids because tissue culture medium usually lacks antioxidants.[33] Micellar BC and LUT were relatively stable after incubation in a cell-free tissue culture environment for 24 hours. This outcome is similar to previous reports showing that α-tocopherol[34,35] and BHT[34] protect carotenoids in the tissue culture environment.

Cellular uptake of BC and LUT from medium containing less than 2 μmol/L carotenoids was proportional to the concentration of carotenoid in medium. The preferential accumulation of BC compared with LUT from micellar medium at most concentrations tested was unexpected. Several possibilities may explain this observation. The greater accumulation of BC may reflect a preferential uptake of this carotenoid compared with LUT. We were unable to measure unidirectional uptake because radiolabeled compounds were not available. However, previous findings suggest that it is unlikely that the transfer of BC, a hydrocarbon carotenoid, from the micelle across the brush border is more efficient than that of LUT, a polar carotenoid.[36] These investigators found that BC is buried deep within a phospholipid-triglyceride droplet, whereas the oxycarotenoid zeaxanthin is located close to the surface and

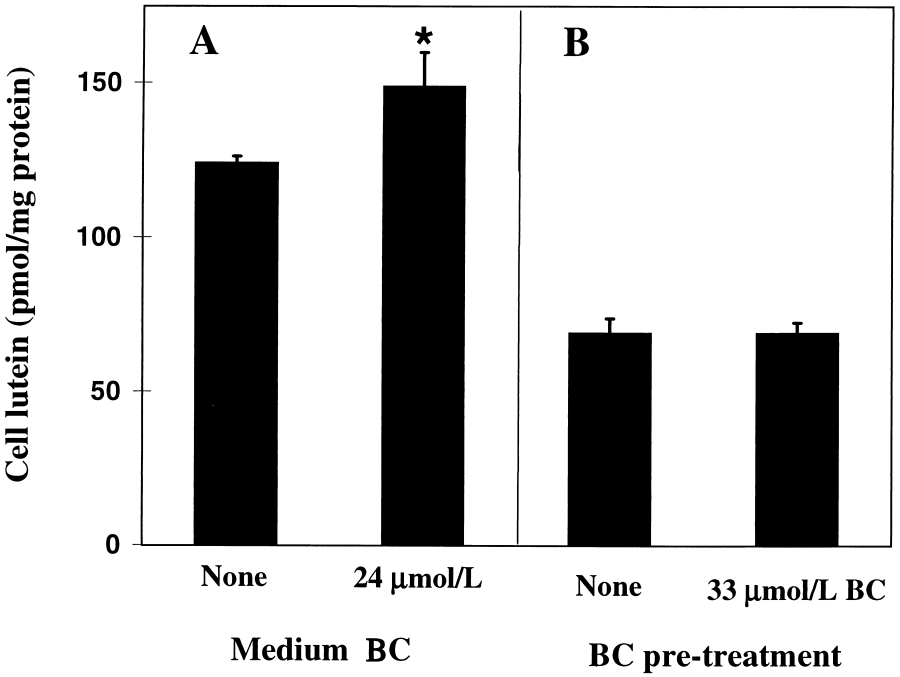

Figure 6 Influence of high levels of extracellular and intracellular β-carotene (BC) on the cellular accumulation of micellar lutein (LUT). *(A)* Confluent cultures were incubated in micellar medium containing 2.9 μmol/L LUT with either 0 (none) or 23.7 μmol/L BC for 8 hours. The asterisk above the error bar denotes that the concentration of cellular LUT was significantly ($P < 0.01$) greater in cultures incubated in medium with LUT plus high levels of BC than in cultures incubated in LUT only. *(B)* Caco-2 cultures were pre-incubated in micellar medium without (none) or with 33 μmol/L BC for 24 hours, resulting in cellular levels of BC that were either less than 1 pmole/mg protein or 432 ± 25 pmoles/mg protein, respectively. Cultures were incubated in fresh micellar medium containing 2.3 μmol/L LUT for an additional 8 hours before determining cellular LUT. Data are means ± SEM of triplicate observations from two experiments.

therefore is readily transferable. Thus, it is more likely that differences in metabolism, retention, and/or spontaneous oxidation of the carotenoids contribute to the apparently greater accumulation of BC in Caco-2 cells. Retinol was not detected in either cells or medium after exposure to micellar BC. It previously was reported that the parent line of Caco-2 cells (i.e., HTB-37) failed to consistently convert BC to retinol.[25] Recently, During et al.[37] reported that the HTB-37 line of Caco-2 cells lacked dioxygenase activity, whereas the PF11 and TC7 clones readily converted BC to retinol. It should be noted that we have taken advantage of the absence of dioxygenase activity in HTB-37 Caco-2 cell line to assess the bioavailability of BC from different foods (manuscript submitted).

We also examined the fates of BC and LUT after pre-loading cultures with these carotenoids. After 36 hours, the cells retained 72% of the initial load of BC. A portion of the residual amount of BC was present in medium, although 16% of that initially accumulated was not present in either cells or medium. It is possible that some BC is lost by spontaneous oxidation or eccentrically cleaved to apocarotenals. We also were unable to detect basolateral secretion of BC via chylomicrons when cells were grown on membrane inserts and exposed to micellar BC and 500 μmol/L oleic acid (data not shown). Thus, it appears that the Caco-2 clones are better suited models for examining the metabolism and basolateral secretion of BC. There was a loss of 50% of previously accumulated LUT from cells after incubation for 36 hours. The maximum concentration of LUT in media represented 21% of that initially present in cells, suggesting that approximately 30% LUT also is metabolized or spontaneously oxidized. The 9-cis and 13-cis isomers of LUT, as well as anhydrolutein, have been detected in human plasma suggesting possible metabolism of LUT during digestion or intestinal absorption.[38] More detailed examination of the handling of intracellular LUT by the parent line and clones of Caco-2 is merited.

Research related to the absorption and metabolism of dietary carotenoids has focused more intensely on BC than on other carotenoids in part because BC is an abundant dietary precursor of retinoids. Accumulation of BC from medium containing higher levels of BC was curvilinear and reached maximum when medium concentrations were 18 μmol/L or greater. This observation is not in agreement with a previous report in which BC accumulation by a rat hybrid intestinal cell line (derived from fusing a spontaneously transformed rat small intestine cell line with rat duodenal epithelial cells) was linear up to a concentration of 25 μmol/L BC.[17] The observed differences in the pattern of BC uptake in these studies may be due to the cell type and the delivery vehicle. Whereas carotenoids were delivered to cells via mixed micelles in the present study, Scita et al.[17] solubilized BC in THF:DMSO for addition to tissue culture medium containing 10% fetal bovine serum. Wei et al.[39] have reported that the accumulation of BC from medium prepared with BC beadlets varied according to cell type. For example, fibroblasts accumulated more BC than liver cells when medium contained a similar concentration of the carotenoid.

Interactions between BC and LUT have been reported in a number of human studies. Several groups of investigators have found decreased plasma levels of LUT after chronic supplementation with BC.[31,32,40] In light of the high concentrations of LUT and zeaxanthin in the eye and their possible role in reducing the occurrence of age-related macular degeneration, chronic supplementation with BC may be problematic. In contrast, individuals fed an algal extract of carotenoids that was enriched in BC showed a preferential increase in LUT in the chylomicron fraction.[41] The results from the present study showed that a relatively high level of extracellular BC

did not adversely affect LUT uptake, suggesting that apical uptake of these carotenoids into Caco-2 cells occurs independently. Similarly, LUT uptake was not altered when cells were pre-loaded with BC. These limited observations suggest that reported interactions likely reflect intracellular or postabsorptive processes rather than the uptake of micellarized carotenoids across the brush border surface of the enterocyte. The parent cell line of Caco-2 (HTB-37) and the TC7 clone[37] appear to be appropriate models to rigorously evaluate the influences of different carotenoids on the metabolism and transfer of individual carotenoids and their metabolites across the basolateral membrane of the enterocyte.

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