Human Lipoproteins as a Vehicle for the Delivery of β-Carotene and α-Tocopherol to HepG2 Cells[1] (44105)

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Abstract. **Highly differentiated human cell lines represent a useful *in vitro* model for the study of carotenoid uptake, metabolism, and function. Carotenoids are usually introduced into tissue culture media either in organic solvents or as micelles, whereas carotenoids are localized in lipoproteins *in vivo*. Initially, the stability of β-carotene and α-tocopherol in micelles and human lipoproteins under standard tissue culture conditions was compared. Recovery of β-carotene and α-tocopherol was 27% ± 2% and 73% ± 2%, respectively, after overnight incubation of micellar β-carotene and α-tocopherol in serum-free medium without cells. This marked loss of β-carotene was attenuated by inclusion of α-tocopherol in micelles. In contrast, recovery of β-carotene and α-tocopherol was 88%–95% when medium containing the total lipoprotein fraction isolated from β-carotene supplemented individuals was incubated overnight without cells. Cellular accumulation of β-carotene and α-tocopherol from medium containing total lipoproteins (1 mg/ml) was proportional to their concentrations in the lipoprotein fraction (r = 0.94 for β-carotene and 0.74 for α-tocopherol). Cells exhibited similar capability of acquiring β-carotene and α-tocopherol from medium containing either low- or high-density lipoproteins. These data show that lipoproteins represent a stable vehicle for delivery of β-carotein and α-tocopherol to HepG2 human liver cells.**

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Dietary carotenoids are absorbed into the blood *via* the lymphatic system and transported in circulation by plasma lipoproteins (1). For example, Johnson and Russell (2) reported that 79%, 8%, and 12% of the β-carotein (βC) in human serum was present in the low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low density lipoprotein (VLDL) fractions, respectively. The types and quantities of carotenoids present in many human tissues generally are reflective of those in plasma, suggesting that there is effective transfer of carotenoids from plasma lipoproteins to tissues (3).

Detailed investigation of the transport and tissue-specific accumulation of carotenoids has been hampered somewhat by the lack of appropriate animal models. Human and animal cells appear to represent useful models for studying the accumulation and metabolism of carotenoids. However, the extreme hydrophobicity and relative instability of carotenoids in oxygen-containing environments make their delivery to cultured cells problematic. Various investigators have added carotenoids to tissue culture media using organic solvents (e.g., tetrahydrofuran, ethanol, and dimethyl sulfoxide), liposomes, water-miscible beadlets, and micelles as vehicles (4). Vehicle alone is usually added to replicate cultures to monitor potential cytotoxicity of the carrier. However, the long-term solubility and stability of the test carotenoids during incubation using standard tissue culture conditions has received minimal attention. More-

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over, it is not clear if the carotenoids partition into lipoproteins, their physiologic carrier, in serum-containing tissue culture medium when introduced in the vehicles indicated above. In the present study, we assessed the influence of standard tissue culture conditions on the stability of βC and α-tocopherol (α-TC) to HepG2 human liver cells. This highly differentiated cell line was selected as the *in vitro* model because it secretes plasma proteins, expresses an inducible cytochrome P450 system, and responds to hormones and cytokines in a manner similar to normal human liver (5, 6). Moreover, HepG2 cells possess specific receptors for apolipoproteins B and E; catabolize LDL, HDL, and apoprotein E–containing particles; and synthesize VLDL, LDL, and HDL (5).

Materials and Methods

Supplies. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated. Tissue culture flasks (T75) and multiwell dishes (3 mm in diameter) were obtained from Costar Corp. (Cambridge, MA). Capsules containing synthetic β-carotene (30 mg/capsule) and placebos were prepared by Hoffmann-LaRoche (Nutley, NJ). Echinenone, βC, and tocol were gifts from Dr. J. C. Smith, Jr., USDA Beltsville Human Nutrition Research Center.

Subjects. Five healthy, normolipemic adult males (25–47 years of age) in our laboratory volunteered to participate in this study, which was approved by The University of North Carolina at Greensboro Human Studies Institutional Review Board. Subjects ingested either a βC supplement (60 mg/day; $n = 2$) or placebo ($n = 3$) at two meals per day for 4 weeks. Blood samples were collected by a trained phlebotomist at the beginning and the end of the 4-week supplementation period from placebo- and β-carotene–supplemented subjects. Additional blood samples were collected at 2 and 4 weeks post supplementation from βC-supplemented subjects to obtain lipoproteins containing different concentrations of βC. Eight weeks after the completion of the first trial period, the study was repeated with the identical subjects ingesting the βC supplements or the placebo. Blood samples were collected at the beginning and the end of the 4-week supplementation period and at 2 week post supplementation. In the second study, total lipoproteins were fractionated to obtain LDL and HDL.

Isolation of Lipoproteins. Fasting blood samples were collected by venipuncture into vacutainer tubes containing Na_2-EDTA (1 m*M*) as anticoagulant. Plasma was separated from whole blood by centrifugation at 1,500*g* fo 25 min at 4°C. Lipoproteins were isolated from the plasma as previously described (7). Briefly, the density of plasma ($d = 1.006$ kg/l) from each subject was elevated to 1.21 g/l by addition of solid potassium bromide (326.5 g/l). Samples were centrifuged (Beckman L7-65 ultracentrifuge and 50.3 Ti rotor) at 114,000*g* for 40 hr at 18°C to flotate all lipoprotein particles. The total lipoprotein fraction was collected and its density readjusted to 1.21 g/ml with potassium bromide. Aliquots (1.8 ml) were transferred to centrifuge tubes, overlaid with 4.3 ml of saline containing 0.01% EDTA and centrifuged as above for 4 hr to isolate VLDL, LDL, and HDL fractions. The lipoprotein fractions were collected using syringe/needle assemblies, transferred to glass tubes, and stored in the dark under nitrogen gas at 4°C for a maximum of 2 weeks. Prior to use in experiments, lipoprotein samples were desalted by passing through a gel filtration column (Econo-Pac 10DG; Bio-Rad, Hercules, CA) packed with a polyacrylamide gel matrix with a 6-kDa pore size and equilibrated with phosphate buffered saline, pH 7.5. Protein levels of total lipoprotein (LP) and of LDL and HDL fractions were determined according to Markwell *et al.* (8).

Cell Culture. HepG2 cells were purchased from American Type Culture Collection (ATCC, Rockville, MD), and stock cultures were maintained in T75 flasks. For experiments, cells were seeded in multiwell plastic dishes (35 mm in diameter) and incubated in a humidified atmosphere of air/CO_2 (95:5 v/v) at 37°C. HepG2 cultures (passages 80–90) were grown in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 1 m*M* pyruvate, 2 m*M* glutamine, 50 mg/l gentamicin, 0.5 mg/l fungizone, and 15 m*M* N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), pH 7.0 (complete medium). Medium was replaced every other day, and experiments were conducted with cultures at 1–3 days post confluency.

Micellar Preparations of βC and α-TC. The procedure used to prepare mixed micelles containing either βC or α-TC was a modification of that described by El-Gorab and Underwood (9) and has been described in detail elsewhere (10). Briefly, appropriate volumes of stock solutions of the following compounds in organic solvents were placed in glass bottles to provide the indicated concentrations after dilution with culture medium. *L*-α-phosphatidylcholine (16.7 μ*M*), monoolein (100 μ*M*), oleic acid (33.3 μ*M*) and either βC or α-TC (15 μ*M*). In one study, micelles containing both βC and α-TC were prepared. After organic solvents were evaporated with a gentle stream of nitrogen, complete cell culture medium containing 5 m*M* sodium taurocholate was added to bottles which were then transferred to a water bath sonicator (Sonic Systems, Inc., Newton, PA) at room temperature. The mixture was sonicated for three cycles of 5 min each. Samples were filter sterilized (SCFA syringe filter with 0.22-μm pore; Nalgene, Rochester, NY) to remove material that was not solubilized before incubating overnight in plastic multiwell dishes. The morphological appearance of the monolayer and the integrity of

the cell surface were not altered by the presence of the micelles in the culture medium (10).

In Vitro Stability of βC and α-TC. The influence of standard cell culture conditions on βC and α-TC was assessed by incubating complete medium containing either micellar preparations or total lipoproteins (1 mg protein/well) in tissue culture dishes without cells for 20 hr. The total lipoprotein fraction was prepared from βC-supplemented subjects (n = 2) during each of the two trial periods. Aliquots of medium containing either micelle or total lipoprotein fraction were extracted and analyzed as described below immediately after filter sterilization and after incubation for 20 hr at 37°C in a humidified atmosphere at air/CO_2 (95:5 v/v). Each test sample was incubated in triplicate wells.

Cellular Accumulation of βC and α-TC. Carotenoids and α-tocopherol were introduced into confluent cultures of HepG2 cells by using lipoproteins as vehicles. Experiments were initiated by overnight incubation of confluent cultures in serum-free medium containing 0.1% ITS (insulin, transferrin, and selenium; Collaborative Research, Bedford, MA) to increase expression of lipoprotein receptors on the cell surface (5). Monolayers were washed and incubated with 1 ml of serum-free MEM containing 0.1% ITS (v/v) and either the indicated lipoprotein fraction or PBS vehicle (control). The concentrations of total LP, HDL, and LDL were 1.0, 1.0, and 0.5 mg protein/ml, respectively. The lower concentration of LDL protein was due to the limited availability of this material. Pilot studies showed that incubation of HepG2 cultures with total lipoprotein concentrations as high as 3 mg/ml did not adversely affect morphology or cellular integrity of the monolayer. After incubation of cultures in lipoprotein-containing medium for 20 hr, monolayers were washed twice with Hanks' balanced salts solution at 0°C, collected in 1 ml of 0.1 mM potassium phosphate buffer (pH 7.5) containing 10% ethanol with 0.45 mM butylated hydroxytoluene, and sonicated for 3–5 sec using a Sonic Dismembranator at 60% power output (Fisher Scientific, Pittsburgh, PA). Aliquots (500 μl) of the sonicate were diluted with an equal volume of ethanol (500 μl) containing either echinenone (0.2 μg/ml) or tocol (25 μg/ml) as internal standards for analysis of carotenoids or α-tocopherol, respectively. Hydrophobic materials were extracted into hexane, dried under nitrogen, and reconstituted in the appropriate mobile phase for high-performance liquid chromatography (HPLC) as detailed below.

HPLC Analysis. βC and α-TC were analyzed by HPLC (Model HP-1090; Hewlett-Packard, Avondale, PA) with a UV/visible dual beam photometer with wavelengths set at 450 nm for carotenoids (11) and 292 nm for α-tocopherol (12). The column was a Microsorb-MV 5-μm 25 × 0.46-cm diameter C-18 ODS (Rainin, Woburn, MA) and was protected by a C-18 Adsorbosphere, 5-μm particle size, 0.75 × 0.46-cm guard column (Alltech, Deerfield, IL). The mobile phase consisted of 70:20:10 (v/v/v) acetonitrile (with 0.13% triethylamine):methylene chloride:methanol (with 0.01% ammonium acetate) at a flow rate of 1.7 ml/min for quantification of carotenoid concentrations. The mobile phase for quantification of α-TC was methanol at a flow rate of 2.5 ml/min. The column temperature was maintained at 20°C using a 40-cm HPLC water jacket (Alltech, Deerfield, IL). Data were calculated based on external standards that were >94% pure as verified by HPLC. The limits of detection for β-carotene and α-tocopherol were 1 and 8 pmol, respectively.

Table I. Concentrations of β-Carotene in Human Plasma and Lipoproteins after Supplementation with β-Carotene or Placebo for 4 Weeks

Sample	Baseline	4-Week supplement	
		β-Carotene	Placebo
Plasma	0.37 ± 0.04	5.12 ± 0.81[a]	0.39 ± 0.05
Total lipoproteins	0.41 ± 0.12	4.71 ± 0.31[a]	0.46 ± 0.07
VLDL	0.03 ± 0.01	0.32 ± 0.06[a]	0.02 ± 0.01
LDL	0.35 ± 0.03	3.44 ± 0.81[a]	0.38 ± 0.03
HDL	0.12 ± 0.04	1.41 ± 0.30[a]	0.12 ± 0.04

Note. Distribution and concentrations of βC (μM) in human plasma and lipoproteins were determined in plasma samples collected from healthy male volunteers before (n = 5) and after supplementation with 60 mg/d βC (n = 2) or placebo (n = 3) for 4 weeks in two independent studies. Concentrations of βC were determined by HPLC as described in Materials and Methods. Because the responses to treatments were similar in the two studies, data were pooled, and values represent the mean ± SEM for βC- (n = 4) and placebo-supplemented subjects (n = 6).

[a] Significant ($P < 0.05$) differences between means at baseline and after 4 weeks.

Miscellaneous Analyses. Triacylglycerol and cholesterol levels in plasma and lipoprotein fractions were determined using kits from Sigma Chemical Co. The protein content of cells were determined by a modification of the method by Lowry *et al.* (13) using bovine serum albumin as a standard.

Statistical Analysis of Data. Student's t test was used to determine differences between means. Differences were considered significant at $P < 0.05$.

Results

Our initial aim was to generate βC-enriched lipoproteins *in vivo* by supplementing the diet of several subjects with 60 mg of βC/day. Two independent studies were conducted with the same individuals ingesting either the βC supplement (n = 2) or the placebo (n = 3). Because the response to the treatment was similar in both studies, data have been pooled for presentation. The concentrations of βC and α-TC in plasma and total lipoproten fractions of subjects at baseline and after 4 weeks of supplementation with either βC or placebo are shown in Tables I and II. After 4 weeks of supple-

Table II. Concentrations of α-Tocopherol in Human Plasma and Lipoproteins after Supplementation with β-Carotene or Placebo for 4 Weeks

Sample	Baseline	4 Week supplement	
		β-Carotene	Placebo
Plasma	48.6 ± 6.0	48.6 ± 12.6	48.2 ± 5.3
Total lipoproteins	42.3 ± 10.5	51.6 ± 5.1	42.7 ± 7.1
VLDL	7.8 ± 2.4	10.2 ± 0.2	11.5 ± 4.9
LDL	22.0 ± 2.4	20.1 ± 0.2	17.8 ± 2.7
HDL	15.9 ± 0.4	17.2 ± 3.5	17.6 ± 5.0

Note. Distribution and concentrations of αTC (μ*M*) in human plasma and lipoproteins fractions were determined in plasma samples collected from healthy male volunteers before ($n = 5$) and after supplementation with 60 mg/d βC ($n = 2$) or placebo ($n = 3$) for 4 weeks. Concentrations of α-TC were determined by HPLC as described in Materials and Methods. Data are pooled means ± SEM for βC- ($n = 4$) and placebo-supplemented subjects ($n = 4$). Mean levels of α-TC were not significantly ($P > 0.05$) altered by supplementation.

mentation with βC, plasma levels of the carotenoid were 12-fold higher than those in subjects ingesting the placebo. The concentration of βC in each lipoprotein fraction increased proportionally in response to dietary supplementation. The relative distribution of βC among the lipoprotein fractions was similar in subjects at baseline and after 4 weeks of supplementation (viz., 67% ± 2%, 25% ± 3%, and 8% ± 2% in LDL, HDL, and VLDL, respectively). βC concentrations in the plasma and lipoprotein fractions of subjects ingesting placebo for 4 weeks were not significantly different ($P > 0.05$) from baseline levels.

The concentrations of α-TC in plasma were not significantly altered ($P > 0.05$) by βC supplementation for 4 weeks (Table II). α-TC was associated primarily with LDL (43% ± 5%), with lesser amounts in HDL (36% ± 1%) and VLDL (21% ± 2%). Plasma levels of triglycerides and cholesterol and of cholesterol among lipoprotein fractions were not altered by supplementation with βC (data not shown).

We next compared the stability of βC and α-TC in serum-free tissue culture medium after their addition in lipoproteins and micelles. The preparations were incubated for 20 hr using standard tissue culture conditions (viz., humidified atmosphere of air:CO_2 in sterile plastic multiwell dishes without cells). Only 27% and 73% of the initial concentration of βC and α-TC, respectively, were present in medium containing micelles with one of the test compounds (Table III). In contrast, 88% and 94% of the initial concentrations of βC and α-TC, respectively, were detected in medium containing the total LP fraction isolated from βC-supplemented subjects. Inclusion of both α-TC and βC in the micellar preparation markedly attenuated the loss of βC (68% recovery), but not α-TC (71% recovery) during the incubation ($P < 0.05$); this suggests that micellar βC was oxidized under standard culture conditions and that α-TC blocked the oxidation of micellar βC.

Basal levels of βC in confluent cultures that were incubated in control serum-free medium for 20 hr were below the level of detection by HPLC (about 1 pmol/mg protein); the level of α-TC in these cells was 6 pmol/mg protein. To assess whether HepG2 liver cells accumulated βC and α-TC from lipoproteins, confluent cultures were incubated in serum-free MEM containing total lipoproteins (1 g protein/l) that had been prepared from individuals supplemented either with βC or placebo. Cellular levels of βC ranged from 1 to 130 pmol/mg protein (Fig. 1, upper panel) and were positively correlated ($r = 0.94$) with the concentrations of βC in medium. The cells accumulated 11% ± 2% of βC in medium. The range of concentrations of α-TC in HepG2 cells incubated in medium containing total LP from test subjects ranged from 53 to 356 pmol/mg protein (Fig. 2, upper panel) and was positively correlated ($r = 0.74$) with the concentration of α-TC in medium. Cells accumulated 14% ± 2% of medium α-TC.

To examine if HepG2 cells preferentially accumulated βC from a specific fraction of lipoproteins, cultures were incubated for 20 hr in medium containing either LDL (0.5 g protein/l) or HDL (1 g protein/l) isolated from plasma of subjects. The concentration of βC in HepG2 cells incubated overnight in medium with the LDL fraction was 3–96 pmol βC/mg protein (Fig. 1, middle panel) and was positively correlated ($r = 0.85$) with the level of carotenoid in the medium. Likewise, cultures incubated in MEM with HDL contained 1–14 pmol βC/mg protein (Fig. 1, lower panel); cellular βC was positively correlated ($r = 0.82$) with the concentration in medium. HepG2 cells accumulated 12% ± 2 and 20% ± 3% of βC introduced into medium *via* LDL and HDL, respectively.

Table III. Effects of Standard Tissue Culture Conditions on Stability of β-Carotene and α-Tocopherol Added to Medium as Either Micelles or Lipoproteins

	% initial concentration	
	Micelle	Lipoprotein
β-Carotene	26.8 ± 2.3	87.7 ± 1.6
α-Tocopherol	73.1 ± 2.1	94.0 ± 1.8
β-Carotene	67.8 ± 2.0	—
plus α-tocopherol	70.9 ± 2.3	—

Note. Complete medium containing either micellar preparations or total lipoproteins from βC-supplemented subjects was incubated in plastic tissue culture dishes without cells in a humidified atmosphere of 95% air/5% CO_2 for 20 hr. The final concentrations of βC and α-TC in medium containing micelles were 1.1 ± 0.3 and 14.1 ± 1.5 μ*M*, respectively. The final concentrations of βC and α-TC in medium containing lipoproteins were 4.7 ± 0.8 and 41.6 ± 5.1 μ*M*, respectively. Aliquots of medium were extracted immediately after preparation and sterilization of medium and after incubation for 20 hr. The concentrations of βC and α-TC were analyzed as described in Materials and Methods. Data are the mean ± SEM for at least triplicate samples from each of two separate experiments.

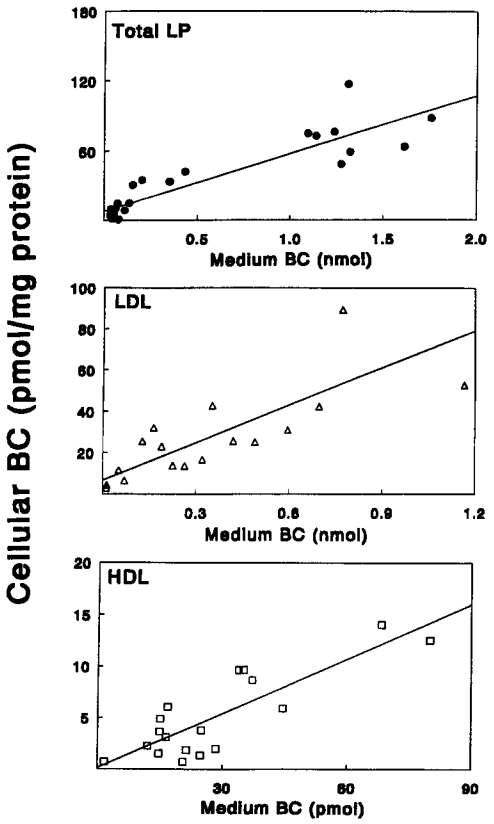

Figure 1. Accumulation of β-carotene (BC) by HepG2 cultures incubated in medium containing human lipoproteins (LP). Confluent monolayers of HepG2 cells were incubated for 20 hr in 1 ml medium containing either 1.0 mg/ml human total lipoproteins (top panel), 0.5 mg/ml human low-density lipoprotein (LDL; middle panel), or 1.0 mg/ml human high-density lipoprotein (HDL; bottom panel) obtained from subjects (n = 5) supplemented with either 60 mg of βC/day or placebo. Plasma samples were collected at the beginning and end of the 4-week supplementation period and at 2 and 4 weeks post supplementation to obtain lipoprotein fractions with different concentrations of βC. After spent medium was removed and monolayers were washed, cultures were harvested, extracted, and analyzed to determine cellular βC. The quantity of βC in the lipoprotein fractions that was introduced at the beginning of the incubation period is shown on the abscissa. Each point represents the mean value for a test sample that was added to three replicate wells.

α-TC levels in cells cultured with LDL ranged from 54 to 333 α-TC/mg protein (Fig. 2, middle panel). Similarly, the concentrations of α-TC in HepG2 cells incubated with MEM containing HDL ranged from 148 to 365 pmol α-TC/mg protein (Fig. 2, lower panel). The concentration of α-TC in HepG2 cells was positively correlated with that in the medium when cultures were incubated with LDL (r = 0.74) and HDL (r = 0.66). HepG2 cells accumulated 8% ± 2% and 11% ± 1% of α-TC added as LDL and HDL to medium, respectively.

Discussion

This study demonstrates that βC and α-TC in human lipoproteins are stable in tissue culture medium and readily accumulated by HepG2 human liver cells *in vitro*. Similar to the report of Micozzi *et al.* (14), we found that βC supplementation markedly increased the concentration of this carotenoid in plasma and total lipoproteins without altering its relative distribution among lipoprotein fractions. We also found that βC was carried predominantly in the LDL fraction and to a lesser extent in HDL and VLDL, as recently reported (2, 15). βC supplementation did not affect either the concentration or the relative distribution of α-TC in lipoproteins, as reported by others (16, 17).

Our initial goal was to generate βC-enriched lipoproteins for delivery of the carotenoid to cells in culture. Because studies regarding *in vitro* uptake of carotenoids by cells have generally failed to consider the relative stability of the test compounds in cell culture medium, we measured the levels of βC and α-TC contained in medium containing either micelles or lipoproteins in MEM before and after incubation under standard tissue culture conditions (37°C and humidified atmosphere of air:CO_2 [95:5]). Both compounds, and particularly βC,

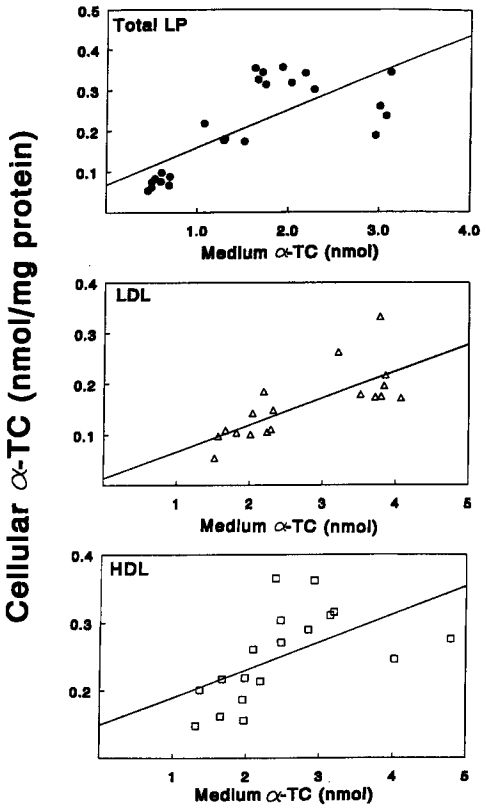

Figure 2. Accumulation of α-tocopherol (α-TC) by HepG2 cells incubated in medium containing human lipoproteins. Confluent cultures of HepG2 cells were incubated for 20 hr in 1 ml medium containing 1.0 mg/ml total lipoproteins (top panel), 0.5 mg/ml low-density lipoprotein (LDL; middle panel), or 1.0 mg/ml high-density lipoprotein (HDL; bottom panel) isolated from subjects supplemented with 60-mg/day β-carotene or placebo for 4 weeks as described in the legend for Figure 1. Aliquots of fresh medium and cell sonicates prepared after 20-hr incubation were extracted and analyzed to determine the concentration of α-TC. The quantity of βC in the lipoprotein fraction that was added to cultures to begin the experiment is shown on the abscissa. Each point represents the mean value for a test sample that was added to three replicate wells.

were less stable when added to tissue culture medium as micelles than as lipoproteins. Inclusion of both α-TC and βC in the micellar preparation decreased the loss of βC during overnight incubation, suggesting that micellar βC is oxidized under standard tissue culture conditions. In contrast, Scita (18) reported that micellar βC was relatively stable in DMEM that was incubated in a humidified atmosphere of air/CO_2 (95:5 v/v) at 37°C for 24 hr. Micellar solutions were prepared by adding βC solubilized in tetrahydrofuran without or with dimethyl sulfoxide to medium containing 10% fetal bovine serum. It is unclear if the tetrahydrofuran used in these studies contained butylated hydroxytoluene, an antioxidant, as a stabilizing agent. Cooney *et al.* (4) have suggested that presence of tetrahydrofuran in these preparations may have solubilized and stabilized βC by forming a molecular "cage" around one or more βC molecules. The composition of both the micelle and the tissue culture medium, as well as the handling of the samples prior to incubation, likely influences the stability of βC in tissue culture medium.

Numerous investigators have characterized the distribution of carotenoids in human lipoproteins (e.g., Refs. 2, 3, and 16). However, we are unaware of published studies evaluating the ability of lipoproteins (viz., HDL and LDL) to deliver βC and other carotenoids to cultured human cells. Thus, our initial studies focused on the accumulation of βC and α-TC from total lipoproteins (LP) by HepG2 cells obtained from subjects supplemented with either βC or placebo. Since most hydrophobic compounds in human plasma are associated with LDL and HDL, the accumulation of βC and α-TC by HepG2 cultures incubated in medium containing these fractions was examined. Our data indicate that confluent cultures of cells readily accumulated βC from medium containing LP, LDL, or HDL as the carrier. The quantity of βC present in cells was proportional to the concentration in medium when either LP fraction served as the carrier. Comparison of our results with several other reports examining βC levels in cells merits comment. Oarada *et al.* (19) reported that FU-5 rat hepatoma cells accumulated 70 pmol βC/mg protein when cultures were incubated for 24 hr in Ham's F-12 medium containing 3.5 m*M* βC (tetrahydofuran vehicle). HepG2 cells incubated in medium containing total lipoproteins (1 g protein/l) isolated from the plasma of subjects supplemented with βC accumulated similar quantities of βC. Peng *et al.* (20) reported 2.2 pmol βC/mg protein in human buccal mucosal cells that had been collected from subjects supplemented with βC for ≤10 days.

Confluent cultures of HepG2 cells express LDL receptors and accumulate and metabolize human LDL (5, 21). Therefore, it is likely that βC accumulation from LDL was mediated at least in part by receptor-mediated endocytosis. Although HDL receptors have been identified on the surface of HepG2 liver cells (5, 22, 23), the mechanism by which they facilitate the uptake of components of HDL remains unclear. The literature suggests there are two distinct processes for uptake of HDL components: one involving endocytotic uptake of apoAI (and thus presumably the HDL particle) and the other involving nonendocytotic uptake of cholesterol esters mostly without uptake of the particle (24). The latter pathway is not limited to the uptake of cholesterol esters since the long-chain dialkyl ester sucrose octaoleate is rapidly accumulated from HDL (24). Studies are needed to define the mechanism(s) of cellular acquisition of carotenoids from lipoprotein fractions, including chylomicron remnants. Also, Leblond and Marcel (25) reported that HDL cholesterol ester is taken up by HepG2 without uptake of the HDL apoprotein. This raises the possibility that the nonendocytotic pathway may be important for the uptake of nonpolar compounds such as βC that are carried in HDL. The high-affinity plasma membrane receptors for HDL in HepG2 cells also appear to mediate the removal of excess intracellular cholesterol (i.e., reverse cholesterol transport) (26). The cellular binding sites for HDL in intact cells are enhanced when cells are loaded with cholesterol or when the rate of cell proliferation is inhibited, as often observed in confluent cultures. It is unknown if βC and other carotenoids may be transferred from the liver cell to HDL by this efflux pathway. Additionally, the extent of metabolism of βC by HepG2 cells accumulated in different lipoprotein fractions is unknown.

In conclusion, this study has demonstrated that βC is relatively stable in tissue culture medium under standard *in vitro* incubation conditions when introduced as a component of lipoproteins. Furthermore, lipoproteins represent effective vehicles for delivery of βC and other lipophilic compounds to HepG2 human liver cells. Thus, the use of the highly differentiated human liver cell line and human lipoproteins represent a physiologically relevant system for investigating the characteristics of carotenoid uptake and metabolism.

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