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β-Carotene Storage, Conversion to Retinoic Acid, and Induction of the Lipocyte Phenotype in Hepatic Stellate Cells

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Abstract Hepatic stellate cells (HSCs) are the major site of retinol (ROH) metabolism and storage. GRX is a permanent murine myofibroblastic cell line, derived from HSCs, which can be induced to display the fat-storing phenotype by treatment with retinoids. Little is known about hepatic or serum homeostasis of β-carotene and retinoic acid (RA), although the direct biogenesis of RA from β-carotene has been described in enterocytes. The aim of this study was to identify the uptake, metabolism, storage, and release of β-carotene in HSCs. GRX cells were plated in 25 cm^2 tissue culture flasks, treated during 10 days with 3 μmol/L β-carotene and subsequently transferred into the standard culture medium. β-Carotene induced a full cell conversion into the fat-storing phenotype after 10 days. The total cell extracts, cell fractions, and culture medium were analyzed by reverse phase high-performance liquid chromatography for β-carotene and retinoids. Cells accumulated 27.48 ± 6.5 pmol/L β-carotene/10^6 cells, but could not convert it to ROH nor produced retinyl esters (RE). β-Carotene was directly converted to RA, which was found in total cell extracts and in the nuclear fraction (10.15 ± 1.23 pmol/L/10^6 cells), promoting the phenotype conversion. After 24-h chase, cells contained 20.15 ± 1.12 pmol/L β-carotene/10^6 cells and steadily released β-carotene into the medium (6.69 ± 1.75 pmol/ml). We conclude that HSC are the site of the liver β-carotene storage and release, which can be used for RA production as well as for maintenance of the homeostasis of circulating carotenoids in periods of low dietary uptake. J. Cell. Biochem. 92: 414–423, 2004.

Key words: carotenoids; retinoids; β-apo-8′-carotenal; Ito cell; liver

High dietary intake of carotenoids is associated with a lowered risk of several types of cancer [Buring and Hennekes, 1995; Russel, 1998]. It is considered that carotenoids have an antioxidant function, and their presence can protect tissues from noxious free radicals [Bast et al., 1998; Dugas et al., 1999]. Alternatively, some carotenoids such as β-carotene are important provitamins of the retinoid group, generating the major vitamin-A active compound, the retinoic acid (RA), which is known to control cell differentiation and is able to prevent displasia and neoplasia of epithelial tissues [Napoli and Race, 1988; Parker, 1996; Napoli, 2000].

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Despite the fact that mammals require retinoids for reproduction, morphogenesis, cell differentiation, and vision, they entirely depend upon the alimentary supply of retinol (ROH) or its precursors such as β-carotene. In view of the fluctuating intake of these compounds, an elaborate system of ROH metabolism and storage is operational in liver. ROH is converted into retinyl esters (RE) in hepatic stellate cells (HSCs), in which it is stored in intracellular lipid droplets [Hendriks et al., 1987; Andersen et al., 1992]. When required, it can be released to maintain the basal level of circulating ROH in the range of 2 μmol/L [Blomhoff et al., 1992; Norum and Blomhoff, 1992]. Circulating RA levels are maintained in the range of 2 nmol/L. Its presence is required for cells such as lymphocytes that cannot convert circulating ROH to RA, but depend upon it for normal function and differentiation [Napoli, 2000]. β-Carotene is absorbed by enterocytes, where it is partly converted to ROH or released into the circulation. Metabolic pathway of β-carotene conversion to ROH is well documented, and the direct biogenesis of RA from β-carotene has also been described [Napoli and Race, 1988; Parker, 1996; Napoli, 2000].

There is a variability in the absorption and effective use of β-carotene as a source of vitamin A. Some individuals show little or no increase in blood β-carotene concentration after a supplementation of β-carotene and are characterized as non- or low-responder. The mechanism underlying the trait is unknown [Johnson et al., 1995; Hickenbottom et al., 2002]. However, for a large group of consumers, circulating β-carotene levels are directly proportional to the ingestion and basal β-carotene levels are maintained during fasting. The origin of this pool of β-carotene is apparently in liver and adipose tissue but its precise location in not known [Thatcher et al., 1998].

In view of the potential importance of β-carotene in the maintenance of normal cell physiology and protection from injury, we addressed the question of its metabolism, storage and release in HSC, which could potentially compensate the fluctuating levels of carotenoids in circulation. A useful model to study β-carotene uptake and metabolism is the GRX cell line, which has been shown to represent the murine HSC, both in their structural and metabolic properties [Borojevic et al., 1985, 1990; Margis and Borojevic, 1989]. GRX cells are myofibroblasts derived from inflammatory fibro-granulomatous lesions in mouse liver that mobilize and activate the adjacent stellate cells [Borojevic et al., 1985]. They express vimentin, desmin, smooth-muscle α-actin, and glial fibrillary acidic protein (GFAP) [Borojevic et al., 1985; Guma et al., 2001]. They can be induced to display the fat-storing lipocyte phenotype by treatment with ROH. Under such conditions, they modify the cytoskeleton, induce synthesis of a full set of carriers and enzymes involved in lipid and retinoid metabolism, as well as receptors involved in ROH uptake [Margis and Borojevic, 1989; Vicente et al., 1998; Fortuna et al., 2001, 2003; Guma et al., 2001; Mermelstein et al., 2001]. The present study addressed the question of the liver uptake, metabolism and storage of β-carotene in HSC using the GRX cell line as a model.

MATERIALS AND METHODS

Reagents

All-*trans*-ROH, all-*trans* ROH palmitate, all-*trans*-RA, β-apo-8′-carotenal, all-*trans*-β-carotene, lipid standards, and the lipotropic dye Oil Red O were purchased from Sigma Chemical Company (St. Louis, MO). Cold water soluble (CWS) β-carotene (1%), and CWS apocarotenal (1%) were kindly provided by Roche (Rio de Janeiro, RJ, Brazil).

Dulbecco's cell culture medium was obtained from Sigma, and fetal bovine serum (FBS) was from Laborclin (Campinas, SP, Brazil). Tissue culture flasks (25 cm^2) and cell scrapers were obtained from Nunc (Roskilde, Denmark), and filter membranes from Millipore (Bedford, MA). Thin layer chromatography (TLC) plates and all the solvents (HPLC grade) were obtained from Merck (Rio de Janeiro, RJ, Brazil). Kodak X-MAT film was from Kodak (São José dos Campos, SP, Brazil). All the chemicals were of analytical grade.

Cell Cultures

GRX and C2C12 cells were obtained from the Rio de Janeiro Cell Bank (Federal University, Rio de Janeiro, RJ, Brazil). GRX cells were plated in 25 cm^2 tissue culture flasks, 5×10^6 cells/flask, and maintained routinely in the Dulbecco's medium supplemented with 5% FBS and 2 g/L HEPES buffer, pH 7.4, under 5% CO_2 atmosphere. Under these conditions, they expressed the myofibroblast phenotype.

Cellular Uptake of Carotenoids During 24–48 h

The response of HSC cells to carotenoids was studied in GRX cells expressing the myofibroblast phenotype. They were incubated for 24 and 48 h in the standard culture medium supplemented with 3 μmol/L CWS β-carotene or with 3 μmol/L CWS β-apo-8′-carotenal, dissolved in water:ethanol (1:1). C2C12 myoblasts were used as controls under similar culture conditions. Saturation of the cellular uptake was monitored in GRX cells incubated with 1, 3, 5 μmol/L CWS β-carotene during the same period. Cell morphology was monitored under a phase-contrast microscope.

Conversion to the Lipocyte Phenotype

GRX cells were incubated for 10 days in the standard culture medium with 3 μmol/L CWS β-carotene, or 3 μmol/L CWS β-apo-8′-carotenal, dissolved in water:ethanol (1:1). After 10 days, GRX cells incubated with β-carotene were transferred into the standard culture medium to monitor β-carotene release. The medium was removed and monolayer was washed twice with buffered saline solution (BSS) after 24, 48, and 72 h. Cell morphology was monitored under a phase-contrast microscope.

High-Performance Liquid Chromatography (HPLC) Analysis of Cell Extracts and Medium

For biochemical analyses, cell monolayers were washed with buffered calcium and magnesium-free saline solution (BSS-CMF), detached with 0.25% trypsin, resuspended in 0.5 ml BSS-CMF and quantified. After addition of 0.5-ml ethanol, cell suspensions were extracted with 1 ml hexane and centrifuged at 10,000*g* for 1 min. The hexane extraction was repeated twice. The extracts were pooled, dried under nitrogen, resuspended in 0.1 ml ethanol, and analyzed by reverse phase HPLC. The cell culture medium (5 ml) was freeze-dried and extracted using the same method. Butylated hydroxytoluene (BHT; 20 μmol/l) was added in all samples, which were stored in liquid nitrogen.

The samples were analyzed using a reverse phase Hypersil ODS column (250 × 4.6 mm; 5 μm particle size), in a Sigma HPLC system. The equipment consisted of a LC-6AD pump, a SPD-10AV UV-Vis spectrophotometric detector and a C-R6A integrator (Shimadzu, Kyoto, Japan). The mobile phase for RA and ROH consisted of a mixture of acetonitrile:methanol: 100 mmol/L ammonium acetate (64:16:20; v/v) [Takeda and Yamamoto, 1994]. The flow-rate was 1.0 ml/min and detection was performed at 340 nm. The mobile phase for ROH and retinyl ester consisted of methanol:hexane (80:20; v/v). The flow-rate was 1.0 ml/min and detection was performed at 326 nm. The mobile phase for β-carotene and β-apo-8′-carotenal was acetonitrile:methylene chloride:methanol (50:20:30; v/v). The flow-rate was 1.0 ml/min and detection was performed at 450 nm [Barua et al., 1993; Wamer et al., 1993].

The identification of retinoids and carotenoids was done by HPLC, using external standard comparison, and by spiking the sample with the external standard and comparison with different chromatographic conditions.

Cell Fractionation

GRX cells expressing the lipocyte phenotype were disrupted and the subcellular fractions were obtained by a modification of the method described by Harrison et al. [1987]. Briefly, the cell monolayers were washed with BSS-CMF, harvested with cell scraper resuspended in 2.5 ml distilled water and 0.3 ml fractionation buffer ($NaHCO_3$ 10×). Cells were disrupted in a Teflon Potter tissue homogenizer and sonicated for 8 min. Tris (100 mmol/L), sucrose (0.25 mol/L), and phenyl-methyl-sulfonyl fluoride (PMSF, 2 mmol/L) were added in the cell suspension. An aliquot of 0.5 ml was separated (total extract), and the cell suspension was centrifuged for 10 min at 1,000*g* at 4°C. The supernatant was collected and centrifuged for 60 min at 100,000*g* at 4°C. The pellet (nuclear fraction) was resuspended in 0.5 ml BSS-CMF. After centrifugation, lipid droplets were floating at the top. The supernatant and the lipid droplet fraction were collected with a Pasteur pipette. All the procedures were performed at 4°C. BHT (20 μmol/L) was added to all fractions and samples were store in liquid nitrogen.

Acetate Incorporation and Lipid Analysis

GRX cells were washed twice with BSS and incubated in serum-free medium with 0.25 μCi/ml [^{14}C]acetate, for the last 24 h of the 12 days of conversion into the lipocyte phenotype with β-carotene. After incubation, the radioactive medium was removed, the cells were washed three times with BSS and the fat droplets were isolated as described above. The lipids were

extracted from droplets by the method of Folch et al. [1957]. The chloroform phase was dried under nitrogen, and the radioactive phospholipids were separated by TLC in hexane:ethyl ether:acetic acid (80:20:1, v/v/v). The radioactive spots were detected by autoradiography of TLC plates, identified by comparison with standards, and quantified by densitometry of the radiographic film.

Statistical Analysis

The presented data are mean values ± standard deviation of three independent experiments done in duplicate (n = 6). Statistical comparisons were carried out by Student's *t*-test or Mann–Whitney *U*-test, and differences were considered significant when $P < 0.05$.

RESULTS

Cellular Uptake of β-Carotene

β-Carotene is a hydrophobic precursor of vitamin A, usually detected in the blood at variable concentrations and dependent upon the dietary supply. Several in vitro studies on β-carotene failed to register the cellular uptake because of its low solubility in culture media. In order to find the appropriate solvent for β-carotene, we tested its cellular uptake with different vehicles. We observed that the levels of β-carotene uptake were higher in cells incubated with medium containing a water-dispersible beadlet formulation (CWS) than in cells incubated with medium containing β-carotene in an organic solvent (data not shown). Similar results have been observed [Wamer et al., 1993]. Hereafter, all the results refer to this vehicle.

Although the beadlet formulation was not a physiological way to supplement carotenoids in culture cells, there are some advantages. Beadlets provide greater accumulation of carotenoids by culture cells than organic solvents, like THF; low reduction and oxidation in medium after 48 h, less than 30%; no cytotoxic effects, even the vehicle alone in concentration range of 0.5–5 mmol/L [Wamer et al., 1993; Shahrzad et al., 2002].

Under the standard culture medium, GRX cells grew in monolayers and had fibroblastoid morphology. They were not fully inhibited by confluence and, after reaching hyperconfluence, they grew in the "hills and valleys" growth pattern. GRX cells can be induced to express the lipocyte phenotype with ROH, RA, or indomethacin, when they decrease their growth rate and progressively accumulate numerous lipid droplets [Margis and Borojevic, 1989; Guaragna et al., 1991; Vicente et al., 1998]. In this study, we show that β-carotene induced also the lipocyte phenotype GRX cells that accumulated refringent fat droplets in their cytoplasm. The induction of lipid storage was not synchronous, and group of cells fully induced into the lipocyte phenotype were present simultaneously with

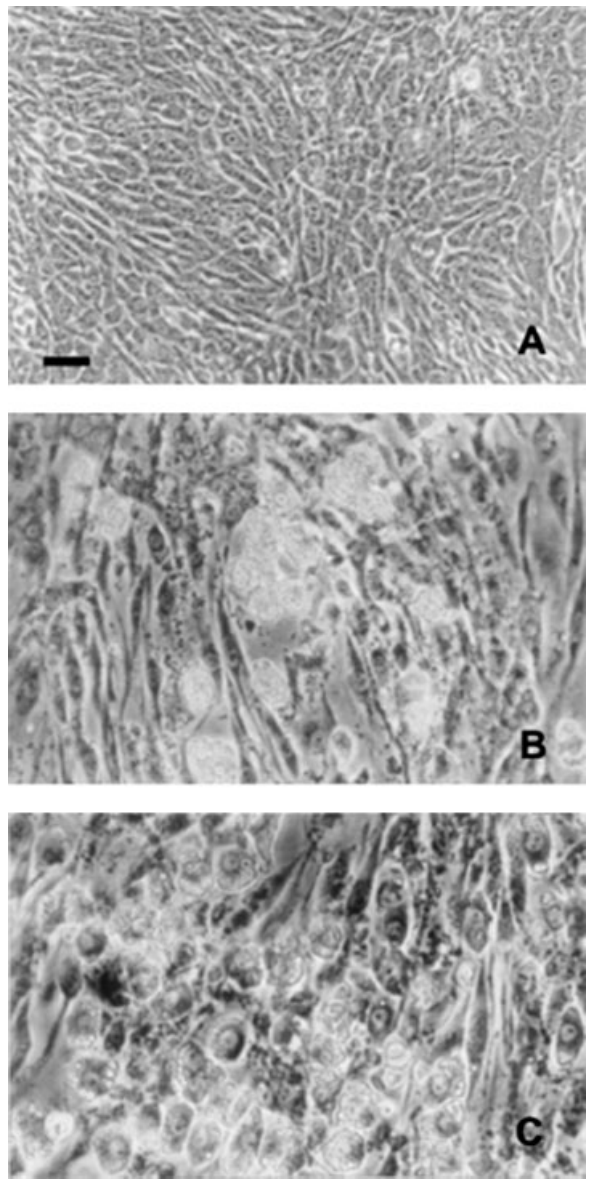

Fig. 1. **A**: GRX in standard culture medium; (**B**) GRX after treatment with standard culture medium supplemented with 3 μmol/L β-carotene during 10 days; (**C**) GRX after treatment with standard culture medium supplemented with 3 μmol/L β-apo-8′-carotenal during 10 days. Scale bar: (A) 5 μm; (B), (C): 10 μm.

cells that had only begun accumulation of lipid droplets. After 10 days of treatment, most cells reached the full lipocyte phenotype (Fig. 1B). The study of dose-dependent response to carotene-mediated induction of the lipocyte phenotype indicated that the minimal concentration required for induction was 3 μmol/L, similar to the data reported for plasma content of humans receiving β-carotene dietary supplementation [Johnson et al., 1995]. The lipid character of these inclusions could be demonstrated by their affinity for the lipotropic dye Oil Red O (data not shown). Although standard culture medium with 5% FBS contains a basal ROH level and RA is undetectable, it maintains GRX cells in the myofibroblast phenotype even after 10 days (Fig. 1A).

GRX cells incubated with 3 μmol/L β-carotene during 48 h showed a time-dependent uptake (Fig. 2). Cellular accumulation of β-carotene increased rapidly during the first 2 h, tended to stabilize at 24 h and further increased slowly during 10 days. In order to test the saturation of the cellular uptake, we incubated myofibroblasts with increasing concentrations of β-carotene during 24–48 h. The uptake in GRX cells was not saturable under the assayed concentrations and time range (Fig. 3). It was proportional to the increase in extracellular content of β-carotene, and GRX cells accumulated β-carotene up to 10 days, concomitantly with accumulation of lipid droplets and full conversion to the lipocyte phenotype.

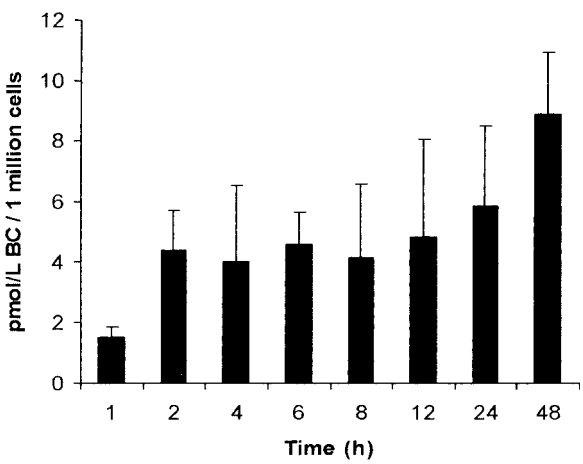

Fig. 2. β-Carotene (3 μmol/L) uptake by GRX during 48 h. One hour was significantly different from 2, 24, and 48 h ($P < 0.01$); 24 h was different from 48 h ($P < 0.01$). The results represent mean values ± SD of three individual experiments.

β-Carotene Metabolism

In order to monitor the β-carotene metabolism, GRX cells were incubated for 10 days and the accumulated carotenes and retinoids were analyzed by HPLC. We observed accumulation of 27.48 ± 6.5 pmol/L β-carotene/10^6 cells, and a significant production of RA, reaching 10.15 ± 1.23 pmol/L/10^6 cells. Retinal and ROH were not observed, nor RE that are readily produced from free ROH in GRX cells [Margis and Borojevic, 1989; Vicente et al., 1998]. These data indicated a direct conversion of β-carotene into RA (Table I). A study of intracellular distribution of retinoids and carotenoids showed that β-carotene was predominantly accumulated in lipid droplets (73%) while RA was found in the nuclear fraction (Table II). The nuclear fraction was a pool of three flasks (5×10^6 cells each). We could not detect RA in other sub-cellular fractions.

In order to monitor the potential exchange of intracellular β-carotene with the culture medium, we loaded GRX cells during 10 days with β-carotene. Cells were subsequently incubated in the β-carotene-free medium containing 5% serum. A rapid initial decrease of intracellular β-carotene was observed in 24 h, but the residual level was roughly maintained for up to 72 h despite the daily change of the culture medium (Fig. 4). These results suggested two mechanisms of β-carotene release, with a rapid mobilization of the high load, and subsequent cell retention of a relatively high fraction of β-carotene. The presence of β-carotene in lipid droplets can be monitored visually, since it turns them yellow. A visible loss of the color was not observed during the 5 days chase, indicating that this carotene fraction was released slowly. In the culture medium, β-carotene was found in a soluble form, reaching 6.69 ± 1.75 pmol/ml, being probably in equilibrium with lipid-carrier proteins in the serum.

Since the absence of retinal and ROH did not support the hypothesis of a central enzymatic cleavage of β-carotene, we questioned whether its metabolism followed the oxidative pathway. We detected the presence of β-apo-8′-carotenal in cells incubated with β-carotene during the first 24 h, confirming this hypothesis (5.90 ± 0.4 pmol/L β-apo-8′-carotenal/10^6 cells). The major product of excentric cleavage found was β-apo-8′-carotenal and it was quantified using internal and external standard by HPLC. We

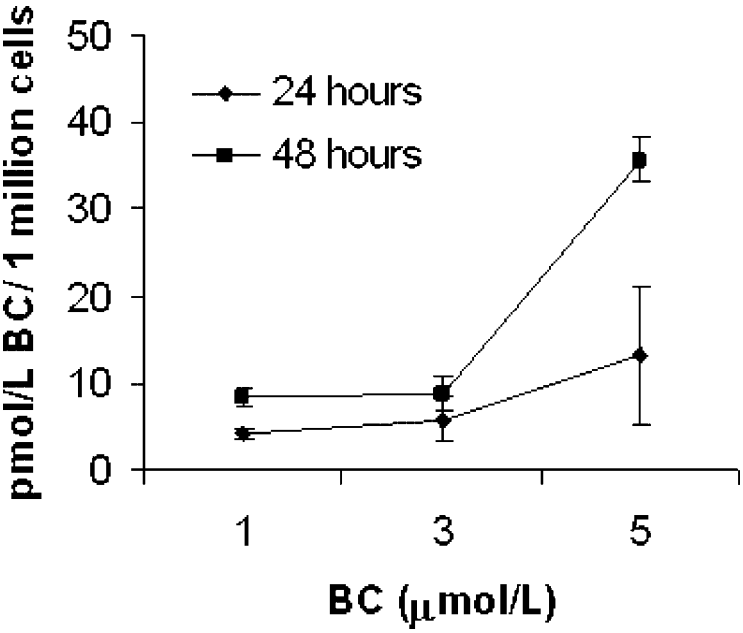

Fig. 3. β-Carotene 1, 3, and 5 μmol/L uptake by GRX during 24 and 48 h. The results shown represent the mean values ± SD of three individual experiments.

also found others product of cleavage but they cannot be quantified. We further supplied GRX cells with 3 μmol/L β-apo-8′-carotenal CWS during 10 days. After 10 days, cells accumulated 529.16 ± 130.2 pmol/L β-apo-8′-carotenal/10^6 cells, corresponding roughly to a tenfold increase as compared to β-carotene uptake. This difference may correspond to the fact that β-apo-8′-carotenal is less hydrophobic than β-carotene. GRX cells also produced 9.75 ± 3.26 pmol/L RA/10^6 cells from β-apo-8′-carotenal after this period. This was similar to cells incubated with β-carotene, indicating that production and accumulation of RA was not dependent upon the availability of the substrate. Cells incubated with β-apo-8′-carotenal converted into the lipocyte phenotype in a similar patter to that observed for β-carotene-induced conversion (Fig. 1C).

β-Carotene and β-Apo-8′-Carotenal Uptake in C2C12 Cells

In order to monitor whether the observed patterns of the uptake of carotenes was specific of stellate cells, we compared GRX cells with C2C12 myoblast cell line originally established from normal adult C3H mouse leg muscle. GRX cells and C2C12 cells were incubated with 3 μmol/L β-apo-8′-carotenal CWS or 3 μmol/L β-carotene CWS during 24 h. Levels of β-carotene and β-apo-8′-carotenal uptake in C2C12 cells were threefold to fivefold lower than in GRX cells (Table III). No retinoids were found in C2C12 cells after incubation with carotenoids.

TABLE I. RA, ROH, RE, and β-Carotene Concentrations (pmol/L/10^6 Cells) After 10 Days Incubation With 3 μmol/L β-Carotene

Component	Concentration (pmol/L/10^6 cells)
RA	10.15 ± 1.23
ROH	Not detectable
RE	Traces
β-Carotene	27.48 ± 6.5

RA, retinoic acid; ROH, retinol; RE, retinyl esters.
The results represent the mean values ± SD of three individual experiments.

TABLE II. β-Carotene and RA Concentrations (%) in GRX Cellular Fractions After 10 Days of Incubation With 3 μmol/L β-Carotene

Cellular fraction	β-Carotene (%)	RA (%)
Cytoplasm	6.9	Not detectable
Membranes	15.8	Not detectable
Lipid droplets	72.9	Not detectable
Nuclei	4.4	100.0

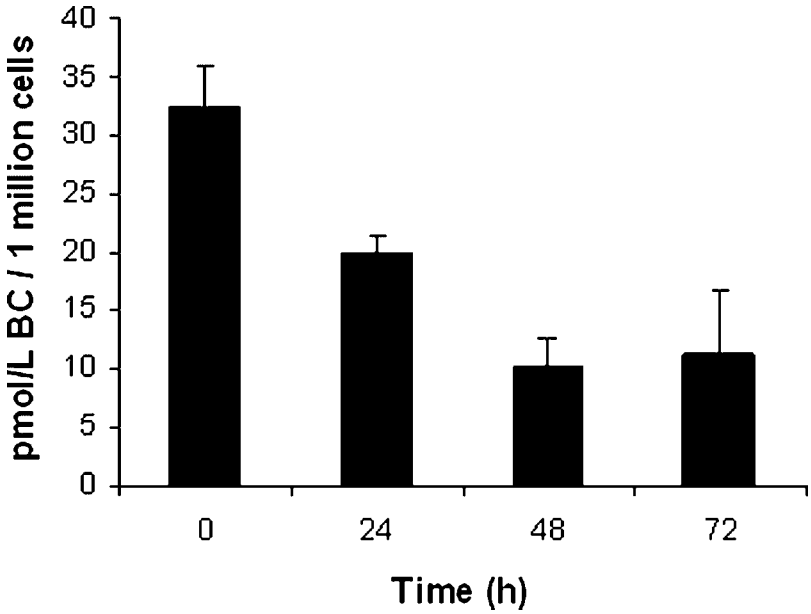

Fig. 4. Intracellular distribution of β-carotene in GRX cells. After treatment with 3 μmol/L β-carotene during 10 days (0 h), the supplemented medium was replaced by standard culture medium without β-carotene during 24, 48, and 72 h. Cells released β-carotene to the culture medium and maintained a basal level of β-carotene. Zero hour was different from 24, 48, and 72 h; 24 h was different from 48 and 72 h ($P < 0.01$). The results represent mean value ± SD of three individual experiments.

Incorporation of Acetate and Lipid Analysis

Full reprogramming of the lipid metabolism is a hallmark of the lipocyte phenotype induction in GRX cells. Incorporation of acetate and the composition of lipid droplets are shown in the Table IV. GRX cells maintained in culture under standard conditions did not accumulate lipids , and incorporation of radiolabeled acetate was undetectable. At 12 days of carotene-mediated induction of the lipocyte phenotype, GRX cells were able to uptake radioactive acetate and synthesize lipid from it. The results showed that triacylglycerols (71.1%) and free fatty acids (18.4%) were the major components of lipid droplets. The overall pattern of lipid accumulation was comparable to that observed for ROH-induced induction of the fat-storing phenotype in GRX cells [Margis and Borojevic, 1989; Guaragna et al., 1991].

TABLE III. β-Carotene and β-Apo-8′-Carotenal Concentrations (pmol/L/10^6 Cells) in the Two Cell Types After 24 h of Incubation With β-Carotene 3 μmol/L or β-Apo-8′-Carotenal 3 μmol/L

Cell type	β-Carotene (pmol/L/10^6 cells)	β-Apo-8′-carotenal (pmol/L/10^6 cells)
M-GRX	12.8 ± 4.54	36.46 ± 0.72
C2C12	2.14 ± 0.2	12.07 ± 0.98

M-GRX, GRX cells expressing the myofibroblast phenotype.
The results represent the mean ± SD of three individual experiments.
All the groups are significantly different ($P < 0.05$).

DISCUSSION

The present study has shown that HSCs can handle carotenoids in a similar way to ROH, in terms of uptake and accumulation. However, a specific pattern of carotenoid metabolism was detected, in which β-carotene is not converted to ROH, but gives origin directly to the RA.

TABLE IV. Composition of Lipid Droplets in GRX

Lipid	% of total incorporation at 12 days
PL	5.1
CHO + DG	2.5
FA	18.4
TG	71.1
AK-DG	2.9

PL, phospholipids; CHO, cholesterol; DG, diacylglycerols; FA, fatty acid; TG, triacylglycerols; AK-DG, monoalkyl-diacylglycerols.
The results represent % of the total incorporation of [^{14}C] in 24 h after 12 days induction with 3 μmol/L β-carotene.

HSCs are considered to be the major site of storage and metabolism of retinoids. Accordingly, we observed that in the model of GRX cells they can also readily uptake large quantities of carotenoids, which could account for the high fluctuation of carotenoid plasma content that follows their dietary ingestion. Lipid-soluble compounds can partition directly into cell membranes, and the non-saturating pattern of carotene uptake in our model is suggestive of this pathway [Borel et al., 1996]. However, the fact that C2C12 cells can uptake a much lower quantity of carotene indicates the presence of additional cellular mechanisms of carotene metabolism and storage. The accumulation of carotenoids in lipid droplets involves an active intracellular transport. In stellate cells, lipid droplets are not surrounded by membranes and a delivery system involving transporter proteins is required for accumulation of hydrophobic molecules in this compartment [Borojevic et al., 1990; Mermelstein et al., 2001]. Cellular ROH-binding protein (CRBP) expression is upregulated in GRX cells that have been induced to express the lipocyte phenotype by ROH [Vicente et al., 1998]. The existence of specific carotenoid-binding protein(s) in stellate cells is not known, but other non-specific transporters such as fatty acid-binding proteins may be involved in the intracellular transport. On the other hand, ROH binding to CRBP is required for the ROH metabolism in stellate cells, since holo-CRBP serves as substrate for enzymes that process ROH in the microsomal subcellular fraction [Ong, 1994; Vicente et al., 1998; Fortuna et al., 2001]. The complex metabolism of β-carotene described here potentially also requires binding of carotene to a specific carrier. Although purification and partial characterization of a carotenoid binding protein from rat and ferret liver has already been described [Okoh et al., 1993; Rao et al., 1997; Lakshman and Rao, 1999], its full characterization has not been achieved, and this is object of ongoing studies.

The existence of two carotenoid pools in terms of their mobilization, described in our model, is compatible with one pool in membranes that is readily equilibrated with the extracellular carotene in the in vitro cell culture, and the other one that is segregated in a specific compartment and requires an active and controlled process to deliver the stored carotene. The latter pool may be in lipid droplets and may maintain a slow steady-state release of accumulated dietary carotenes. This may explain the putative systemic protective role of carotenes against the noxious active radicals, which is expected to be continuously operational despite the irregular dietary intake of carotenoids intercalated by relatively long fasting periods.

The major pathway for generation of retinoids from β-carotene is considered to be the central cleavage by 15–15′ β-carotene monooxygenase into retinal, followed by its reduction to ROH [Parker, 1996]. In mammals, the highest activity of this enzyme was found in the intestinal mucosa and jejunum enterocytes, but similar activity has also been described in liver, lung, kidney, and brain. The enzyme activity was found in the total liver tissue, but its presence in HSCs that are the major site of ROH storage was not determined [Lintig and Wyss, 2001; Wyss et al., 2001]. The eccentric cleavage of β-carotene can produce a series of β-apocarotenals of different chain lengths, such as β-apo-8′-carotenal, all of which can be converted to retinal or oxidized to the corresponding β-apocarotenoic acids. These compounds can subsequently undergo β-oxidation to yield RA [Tang et al., 1991; Wang et al., 1991; Parker, 1996]. A number of experiments demonstrated that RA is a metabolite of β-carotene in different tissues: intestinal mucosa, kidney, lung, testicules, liver, and adipose tissue [Napoli and Race, 1988; Wang et al., 1992, 1993; Napoli, 1993; Hébuterne et al., 1996; Wang and Krinsky, 1997]. Recently, a mammalian enzyme responsible for eccentric cleavage was identified and molecularly characterized. This enzyme catalyzed the eccentric cleavage at the 9′,10′ double bound of β-carotene and resulting in the formation of β-apo,10′-carotenal [Kiefer et al., 2001].

The present study has shown that GRX cells convert β-carotene probably by oxidative eccentric cleavage into RA without producing ROH and retinal. RA production in stellate cells follows thus two pathways, dependent upon its origin in ROH or β-carotene. Both pathways result in the full induction of the lipocyte phenotype, which depends directly upon RA and its interaction with nuclear RA receptors. Metabolism of lipids was similar in lipocytes induced by ROH or β-carotene. This confirmed our previous results on independence of the lipid metabolism pattern of the induction pathway, since increase of lipid synthesis is also similar in ROH and insulin-indomethacin induced lipocytes [Guaragna et al., 1991].

Conversely, induction of specific carrier-proteins may be different since carotene storage and release are not coupled with ROH production and storage.

Taken together, our study indicates that HSCs can have a specific role in storage and release of carotenoids, which can be used for RA production as well as for maintenance of the homeostasis of circulating carotenoids in periods of low dietary uptake.

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