Eur J Nutr (2009) 48:261–268
DOI 10.1007/s00394-009-0001-6

ORIGINAL CONTRIBUTION

Lycopene isomerisation and storage in an in vitro model of murine hepatic stellate cells

Anderson J. Teodoro · Daniel Perrone · Renata B. Martucci · Radovan Borojevic

Received: 26 July 2008 / Accepted: 28 January 2009 / Published online: 17 June 2009

Abstract
Background Lycopene is a carotenoid whose biological activities and protective effect on prostate and breast cancer have been described, but little is known on its extra-intestinal metabolism and storage. While most alimentary lycopene is in *all-trans* configuration, in animal and human tissues approximately half of the lycopene is in *cis* isoforms.
Aim of study Our object was to monitor the capacity of storage, isomerisation, and intracellular localization of *all-trans* and *cis* lycopene in hepatic stellate cells, which are the major sites of metabolism and storage of retinoids and carotenoids in the body.
Methods We used the GRX cell line representative of murine hepatic stellate cells, incubated with 1–30 μM lycopene in culture medium. Analysis was done by high-performance liquid chromatography.
Results Lycopene was able to induce expression of the lipocyte phenotype and it was internalized into GRX cells. Its cellular release only occurred in presence of albumin with a rapid initial decrease of intracellular lycopene. A corresponding increase in the culture medium was observed at 24 h. *All-trans*, *13-cis* and *9-cis* lycopene isoforms were identified in all the cell compartments. The membrane fraction contained the major part of lycopene, followed by the cytoplasmic fraction, lipid droplets and nuclei. The ratio between *all-trans* and *cis* isomers was approximately 2/1 in the majority parts of cell compartments.
Conclusions This study identified a novel hepatic cell type able to store and isomerise lycopene. Liver can contribute to the serum and tissue equilibrium of *cis/trans* isomers of lycopene, and to participate in storage of lycopene under high extracellular concentration such as observed after the alimentary input.

Keywords Lycopene · Metabolism · Isomerisation · Liver · Hepatic stellate cells

A. J. Teodoro · D. Perrone · R. B. Martucci · R. Borojevic
Laboratório de Bioquímica Nutricional e de Alimentos, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

A. J. Teodoro · D. Perrone · R. B. Martucci · R. Borojevic (✉)
Laboratório de Bioquímica Nutricional e de Alimentos, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Caixa Postal 68021, Rio de Janeiro, RJ 21941-970, Brazil
e-mail: radovan@hotmail.com

Introduction

Lycopene is an acyclic C_{40} nonpolar carotenoid, present in several nutritional sources such as tomato, watermelon, guava and apricots [3]. Although lycopene is not a provitamin-A, recent studies have shown its multiple biological activities including decreased prostate cancer risk [18], inhibition of cell proliferation, migration and invasion in breast, endometrial and liver carcinoma cells [1, 8–10, 24, 32], and prevention of mutagenesis and chromosome instability [27, 34]. The mechanism(s) of these activities are not fully understood.

Metabolism and biotransformation of carotenoids have been investigated in vivo, but most studies were dedicated to β-carotene and only a few ones to lycopene. Although *cis* isomers reach more than 50% of the total lycopene in human serum and tissues, lycopene is found in most food sources as the *all-trans* isomer [3, 12, 38]. Isomerisation of lycopene in the stomach as a result of the low pH can be

only partially responsible for generation of *cis* lycopene isomers [31]. The high tissue concentration of *cis* isomers has raised the question of whether they may be more bioavailable and/or more bioactive than the *all-trans* isoforms. This was supported by studies that showed preferential uptake of *cis* isomers in various tissues including enterocytes, followed by the transfer into lymph and the subsequent release into the systemic circulation [2]. Alternatively, lycopene isomerisation may occur within the tissues in which it is incorporated. Significant discrepancies among tissues were observed both for uptake, isomerisation and further metabolism of ^{14}[C]lycopene in rats, the highest values of ^{14}C at all the studied periods (up to 168 h) being found in liver [43]. Proportion of *cis* lycopene in liver was reported to be close to 50% and 20% in ferrets and rats, respectively [14]. Hormones and nutritional status were shown to interfere with the lycopene *trans/cis* ratio in rats [4].

Liver is the major site of metabolism and accumulation of retinoids and carotenoids, where they are mostly accumulated in lipid droplets of hepatic stellate cells, which are specialized in storage and release of endogenous and exogenous lipid-soluble mediators, vitamins and hormones [35]. Since these cells represent <1% of the liver volume, their intracellular content of carotenoids and retinoids can be very high. Retinol conversion to retinoic acid in hepatic stellate cells has been documented, and they can release both retinol and retinoic acid into the circulation [1]. We have recently shown that hepatic stellate cells can also uptake and accumulate β-carotene and convert it directly to retinoic acid [29]. Since liver is also the major site of lycopene accumulation in lycopene-supplemented animals, it is not surprising that the presence of apo-8′-lycopenal and apo-12′-lycopenal in rat liver has been described, representing putative lycopene metabolites [17].

A useful model to study metabolism of hepatic stellate cells is the established murine GRX cell line, which has been shown to represent the murine hepatic stellate cells, both in their structural and metabolic properties [16, 21, 23]. GRX cells are myofibroblasts derived from inflammatory fibro-granulomatous lesions in liver, which mobilize and activate the adjacent hepatic stellate cells in mice and in humans [5, 7, 11]. Their cytoskeleton contains vimentin, desmin, smooth-muscle α-actin and glial fibrillary acidic protein [21]. They can be induced to display the fat-storing lipocyte phenotype of resting hepatic stellate cells by treatment with retinol, retinoic acid, β-carotene or indomethacin [6, 28, 29]. 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 retinol uptake [15, 16, 40].

The object of the present study was to determine the capacity of storage, intracellular localization and isomerisation of *all-trans* lycopene in murine hepatic stellate cells.

Materials and Methods

Reagents

All-trans lycopene and dimethylsulfoxide were purchased from Sigma Chemical Company (St. Louis, MO, USA). Water-soluble (WS) lycopene 10% (containing sucrose, corn starch, fish gelatine, lycopene, corn oil, ascorbyl palmitate and DL-alpha-tocopherol) was provided by Roche (Rio de Janeiro, RJ, Brazil). Dulbecco's cell culture medium and bovine serum albumin were obtained from Sigma, and fetal bovine serum (FBS) from Laborclin (Campinas, SP, Brazil). Tissue culture flasks and cell scrapers were obtained from Nunc (Roskilde, Denmark). All the solvents [high-performance liquid chromatography (HPLC) grade], toluene and ethanol were obtained from Merck (Rio de Janeiro, RJ, Brazil). All the chemicals were of analytical grade.

Cell cultures

GRX 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, 5.0×10^6 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. In order to induce the lipocyte phenotype, cells (10^4 cells/cm^2) were incubated for 10 days in the standard culture medium with 1–30 μM WS lycopene dissolved in water at 50 °C. We also monitored the incubation of *all-trans* lycopene dissolved in toluene, ethanol and dimethylsulfoxide. All the solutions were prepared every day and tests were done in dark to maintain lycopene stability.

The uptake of lycopene was monitored in GRX cells expressing the myofibroblast phenotype, observed in vivo during the low retinoid and carotenoid alimentary input. Standard culture medium was supplemented with WS lycopene from 1 to 30 μM during the selected periods. Saturation of the cellular uptake was monitored in GRX cells incubated with 10, 20 and 30 μM during 72 h. Cell morphology was observed under a phase-contrast microscope.

Lycopene release was monitored after 10-days load in 3 μM lycopene-containing medium, in fresh culture medium without lycopene, supplemented or not with 6% bovine serum albumin (BSA), for up to 96 h.

Cellular protein content was monitored by BCA protein assay kit (Pierce, USA) based on the method described by Smith [37], using BSA as standard.

High performance liquid chromatography analysis of cell extracts

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 twice with 1 mL hexane and centrifuged at 10,000*g* for 1 min. The extracts were pooled, dried under nitrogen, resuspended in 0.1 mL methanol and analyzed by reverse phase HPLC. Lycopene solution and cell culture medium with lycopene were extracted using the same method in order to test its stability and purity. Butylated hydroxytoluene (BHT; 20 μM) was added to all samples, which were stored in liquid nitrogen.

In order to quantify *all-trans* and *cis* lycopene, peak areas were monitored after their separation in HPLC, 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 *all-trans* and *cis* lycopene was a mixture of acetonitryle:methanol (1:1 v/v). The flow-rate was 1.0 and 1.5 mL/min and detection was performed at 472 nm [39]. The quantification of *all-trans* lycopene and *cis* isomers was done by retention time of HPLC, using external standard of *all-trans* lycopene, by the method reported by Yeum et al. [42].

In order to confirm and identify *all-trans* and *cis*-lycopene isomers, we used a reverse phase Magic C30 (150 × 2.0 mm; 5 μm, 100 Å; Michrom Bioresources, Auburn, CA, USA) in a Sigma HPLC system. The equipment consisted of SCL-10A, LC-10AD pump, FCV-10AL mixer, DGU 14A dryer, SPD-M10A alignment detector, and Rheodyne injector with a 20 μL loop, controlled by LCMS solution program. The mobile phase for *all-trans* lycopene and *cis* isomers consisted of a mixture of MeOH (0.1% TEA)/TBME (0.1% TEA) (1:1, v/v) [33]. The flow-rate was 0.5 mL/min and detection was performed at 300–600 nm. The identification of *all-trans* lycopene and *cis* isomers was done by elution order and UV–vis spectra; they were compared to the published data [34, 42].

Cell fractionation

GRX cells expressing the lipocyte phenotype were disrupted, and subcellular fractions were obtained by a modification of the previously described method [22]. Briefly, the cell monolayers were washed, harvested and disrupted in a Teflon Potter tissue homogenizer followed by sonication. Tris (100 mM), sucrose (0.25 mM) and phenyl-methyl-sulphonyl fluoride (PMSF, 2 mM) were added to 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 stored in liquid nitrogen.

Statistical analysis

The presented data are mean values ± standard error of three independent experiments done in duplicate ($n = 3$). Statistical comparisons were carried out by ANOVA and post hoc Tukey's test using Graph Pad Prism 4.0 and Statistical 6.0 program. The differences were considered significant when $P < 0.05$.

Results

Cellular uptake of lycopene and phenotype conversion

Several in vitro studies on lycopene failed to register its cellular uptake in a broad range of concentrations because of its low solubility in culture media. We tested its uptake with different vehicles, and observed that the levels of lycopene uptake were higher in cells incubated with medium containing a water-soluble beadlet formulation (WS) than in cells incubated with medium containing lycopene in different organic solvents (data not shown). Previous studies reported that lycopene formulated as 10% WS granules was not toxic, the compound contained antioxidants that prevented its degradation, and it was accumulated in liver [30]. No cytotoxic effects were observed with the vehicle alone in the range of 0.5–5 mM [36, 41]. Hereafter, all the results refer to lycopene with this vehicle.

Under the standard culture conditions, GRX cells grew in monolayer and had fibroblastoid morphology corresponding to the activated stellate cells in liver tissue. They were not fully inhibited by confluence, and after reaching hyperconfluence they grew in the "hills and valleys" growth pattern, typical of the smooth muscle cell lineages. Although standard culture medium with 5% FBS could potentially contain undetectable quantity of carotenoids or retinoids, GRX cells always displayed the myofibroblast phenotype in the presence FBS only, even after 10 days of

culture (Fig. 1a). In presence of 1, 3, and 5 μM lycopene they were induced to express the lipocyte phenotype, accumulating refringent fat droplets in their cytoplasm. As usual, the induction of lipid storage was not synchronous, and groups of cells fully induced into the lipocyte phenotype were present simultaneously with cells that had only begun accumulation of lipid droplets. After 10 days, most cells reached the typical lipocyte phenotype (Fig. 1b–d). The lipid character of cell inclusions could be demonstrated by their affinity for the lipotropic dye Oil Red O (data not shown). In previous studies, we showed that 3 μM β-carotene induced also the lipocyte phenotype, and this condition was used as a positive control [29].

GRX cells were incubated with 3 μM lycopene, and the cell culture medium was replaced every 24 h. Lycopene cell content was monitored from 12 h to 10 days. From 12 to 48 h the basal intracellular lycopene content was constant ($P > 0.05$). From this point on, a linear increase was observed (Fig. 2) ($P < 0.05$). In high lycopene concentrations, the uptake was not dependent upon the extracellular concentration of lycopene. At 24 h the basal level found at 3 μM was observed also in presence of 10, 20 or 30 μM, but at 72 h, when the steep increase of lycopene was already observed, higher levels were found, but they were not proportional to the extracellular lycopene (Fig. 2, insert).

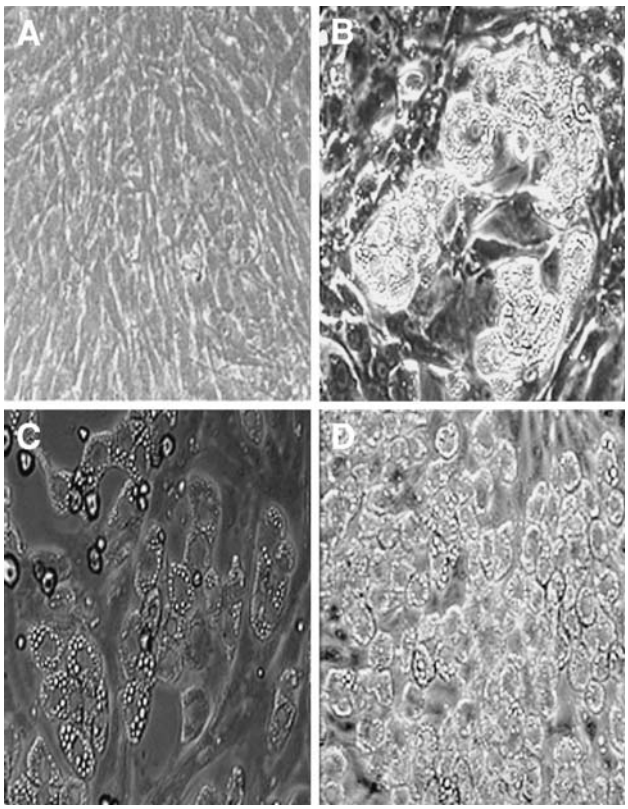

Fig. 1 GRX cells grown in standard culture medium (**a**), GRX cells after treatment with standard culture medium supplemented with 1 (**b**), 3 (**c**) and 5 (**d**) μmol/L *all-trans* lycopene during 10 days. Original magnification ×400

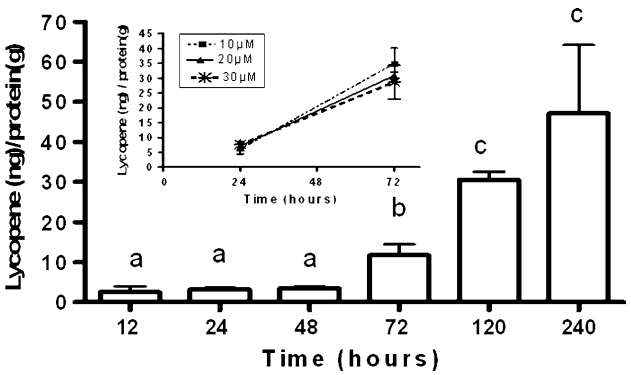

Fig. 2 Cellular uptake of lycopene (ng lycopene/g cell protein) after 10 days incubation of GRX cells in culture media containing 3 μM lycopene. *Bars* represent mean values with standard errors ($n = 6$), $P < 0.05$ for all the fractions. Means with different superscript letter (*a*, *b*, *c*) are significantly different ($P < 0.05$). Cellular uptake of lycopene (ng lycopene/g cell protein) during 72 h incubation of GRX cells in culture media containing 10, 20 and 30 μM lycopene is shown in *insert*. Means with different superscript letter (*a*, *b*) are significantly different ($P < 0.05$)

In order to monitor the potential exchange of intracellular lycopene with the culture medium, we loaded GRX cells during 10 days with lycopene (3 μM). Cells were subsequently incubated in the lycopene-free medium containing 5% serum, containing or not 6% BSA, equivalent to

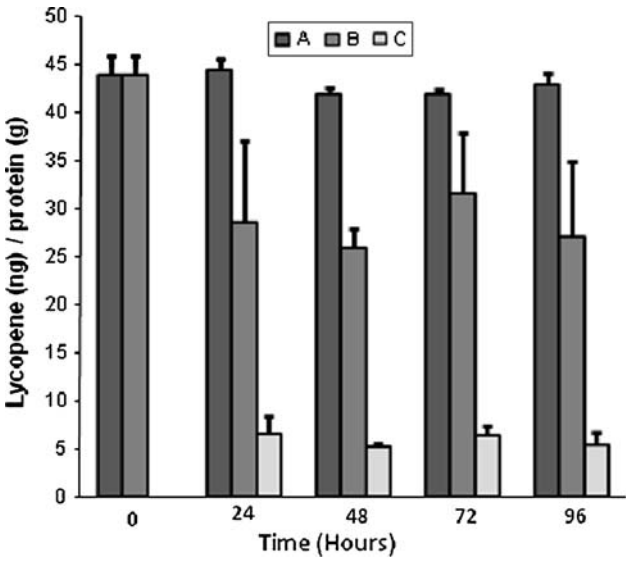

Fig. 3 Lycopene release after 10 days (0 h) load in 3 μM lycopene in fresh culture medium without lycopene, supplemented or not with 6% bovine serum albumin (BSA), for up to 96 h. Lycopene concentration in medium after incubation with 6% BSA (*A*), intracellular lycopene concentration after incubation with 6% BSA (*B*), intracellular lycopene concentration after incubation without BSA (*C*)

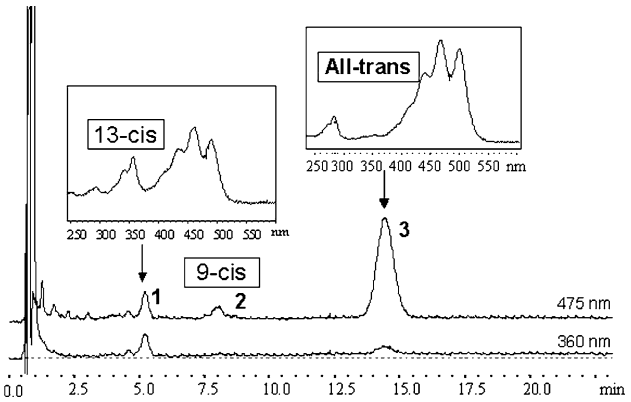

Fig. 4 HPLC-diode array UV/vis GRX cells incubated for 10 days with 3 μmol/L *all-trans* lycopene. The HPLC profiles of the extracts are displayed when monitored at 472 and 360 nm. *Peak 1* corresponds to 13-*cis* lycopene, the UV spectrum is shown in *insert* (*A*). *Peak 3* corresponds to *all-trans* lycopene, the UV spectrum is shown in *insert* (*B*). The peak 2 corresponds potentially to 9-*cis* lycopene

the albumin content in plasma. No release was observed in medium devoid of albumin (Fig. 3a). As expected, in presence of albumin, a rapid initial decrease of intracellular lycopene and a corresponding increase in the culture medium was observed at 24 h, being roughly maintained for up to 96 h (Fig. 3b, c).

Lycopene Isomerisation

The WS lycopene supplied to the culture medium was in the *all-trans* isoform. In order to monitor the lycopene isomerisation, GRX cells were incubated with lycopene-containing culture media for 10 days. We observed three peaks whose identification was done by separation in HPLC followed by analysis in diode UV/vis array (Fig. 4). The major peak (peak 3) obtained by HPLC analysis showed the retention time of 14.5 min expected for lycopene; the UV spectrum absorption was identical to the *all-trans* lycopene standard.

Another peak (peak 1) observed in the cell extracts after 10 days of incubation with lycopene had UV/vis spectra corresponding to the 13-*cis* lycopene [42]. Following the order of elution and UV/vis spectra, we concluded that the peak 2 was 9-*cis* lycopene (Fig. 4). These peaks were not detected in media incubated for 48 h at 37 °C, excluding the possibility of spontaneous isomerisation not mediated by cells, due to light or to oxygen presence (data not shown).

The absolute values of *all-trans* and *cis* isomers of lycopene/g protein in GRX cells incubated with 1–30 μM are presented in the Table 1. The total content of accumulated lycopene increased following the concentration of the extracellular lycopene, although not to the same proportion. However, the *all-trans* lycopene cellular content was quite proportional to its content in the culture medium, indicating that the capacity of the *all-trans* lycopene uptake was not saturated at these concentrations ($P < 0.05$), and its cellular uptake was potentially equilibrated with the extracellular content. Conversely, the quantity of intracellular isomers showed a tendency to reach saturation, and the relative isomers content decreased in relation to the total content of accumulated lycopene ($P > 0.05$). Taken together, these data indicate that the uptake and isomerisation of lycopene in GRX cells have different quantitative controls.

Cell fractionation

In order to monitor the lycopene distribution within the cells, GRX cells were incubated for 10 days with lycopene as described above, and subsequently fractioned to monitor the intracellular localization of lycopene (Table 2).

All-trans and *cis* lycopene isomers were identified in all the cell compartments. The membrane fraction contained the major part of lycopene, followed by the cytoplasmic fraction. The ratio between *all-trans* and *cis* isomers was approximately 2/1 in both. Surprisingly, fat droplets of

Table 1 Concentration of *all-trans* lycopene and lycopene *cis*-isomers in GRX cells after treatment with standard culture medium supplemented with 1–30 μmol/L *all-trans* lycopene during 10 days

Lycopene concentrations (μM)	*All Trans*		13-*cis*		9-*cis*		Total *cis*-isomers[a]		Total lycopene (ng/g)
	ng	%	ng	%	ng	%	ng	%	
1	10.34 ± 1.88[a]	46.08	9.52 ± 6.37[a]	38.68	4.84 ± 3.01[a]	15.24	14.27 ± 8.90[a]	53.92	24.61 ± 7.06 [a]
3	24.26 ± 11.37[b]	65.66	8.07 ± 5.12[a]	20.94	6.21 ± 4.09[a]	13.4	14.28 ± 9.05[a]	34.34	38.54 ± 19.80[b]
5	39.42 ± 9.99[b]	67.19	13.55 ± 10.32[a]	23.50	7.04 ± 0.22[a]	9.31	20.59 ± 14.53[a]	32.81	57.65 ± 14.12[b]
10	49.51 ± 9.31[b]	71.75	10.68 ± 1.23[a]	15.47	8.82 ± 0.36[a]	12.78	19.50 ± 0.87[a]	28.25	69.01 ± 10.18[b]
20	49.70 ± 4.18[b]	78.44	10.90 ± 1.22[a]	17.20	7.81 ± 0.86[a]	12.32	18.71 ± 2.08[a]	29.52	63.36 ± 0.88[b]
30	57.23 ± 11.62[b]	89.90	7.45 ± 1.15[a]	11.70	6.47 ± 0.55[a]	10.16	13.92 ± 1.65[a]	21.86	63.68 ± 11.42[b]

[a] *Cis*-isomers: sum of 13-*cis* and 9-*cis* lycopene isomers

Means with different superscript letter (a, b) are significantly different ($P < 0.05$)

Table 2 Concentration of total lycopene and of *all-trans* and *cis* isomers in GRX cell fractions after treatment with standard culture medium supplemented with 3 μmol/L *all-trans* lycopene during 10 days

Cellular fractions (3 μM)	Total	Distribution (%)	*All-trans*		*Cis*-isomers[a]	
	ng/g		ng/g	%	ng/g	%
Membranes	23.94 ± 13.22	65.24	16.90 ± 2.39	70.01	7.04 ± 0.97	29.99
Cytoplasm	7.79 ± 1.75	21.54	4.97 ± 0.45	64.61	2.81 ± 0.49	35.39
Nuclei	1.76 ± 0.32	4.91	1.44 ± 0.21	81.04	0.32 ± 0,12	18.96
Lipid droplets	3.17 ± 1.99	8.31	1.79 ± 0.60	54.29	1.38 ± 0,45	45.71

[a] *Cis*-isomers: sum of 13-*cis* and 9-*cis* lycopene isomers

GRX cells were not the major site of intracellular lycopene accumulation, in contrast to retinoids and to β-carotene. However, lipid droplets presented the highest relative concentration of isomerised lycopene, with the ratio close to 1/1, indicating a selective accumulation of this lycopene isoform in this cell compartment.

Discussion

The present study provided several sets of information. In accordance with the known function of hepatic stellate cells in uptake, metabolism and storage of retinoids and carotenoids, lycopene was internalized in vitro into GRX cells, and reached high intracellular concentrations. Different from retinol [15], the lycopene uptake was apparently due essentially to its partitioning into the cell membranes, which contained a large part of the cell lycopene. The equilibrium was reached during the fist day of incubation, both in uptake and release of lycopene, and was maintained for at least 3 days.

Surprisingly, from 72 h on, a new mechanism of uptake became operational, steadily increasing the intracellular lycopene content. This is compatible with a synthesis of a new transport protein, induced by a low initial input of lycopene. In contrast to retinol and β-carotene that are essentially accumulated in the stellate cell fat droplets, intracellular lycopene was mostly retained in membranes and cytoplasm. The content of a highly hydrophobic molecule in cytosol requires also its association with a carrier that is at present unknown, and this issue is under study.

The data on uptake and isomerisation of lycopene in high supra-physiological concentrations in the culture medium corroborate this hypothesis. Even under such conditions, the *all-trans* lycopene content was proportional to the extracellular concentration, but the *cis*-isomers did not follow it, indicating that the isomerisation process was saturable. This is compatible with the requirement of the enzyme and/or a cytosolic carrier that presents the lycopene to the enzyme, such as occur in the retinoid metabolism. Recent study showed an alteration in lycopene biodistribution by 15-15′-carotenoid-monoxygenase [26].

The role of the water-soluble carbohydrate carrier used in the culture medium inside the cytoplasm is not probable, since the high membrane content of free lycopene suggested its transport without the carrier. Moreover, the carbohydrates that compose the water soluble formulation are highly biodegradable and if internalized they would be rapidly degraded in the cell. The similar formulation of water-soluble β-carotene used in our previous study did not provide the cytosolic retention of this carotenoid [29].

The relatively low content of lycopene in fat droplets, remaining in the range of 10% of the total cell lycopene, stands in contrast to β-carotene, whose concentration in fat droplets reached more than 70% of the total cell content [29]. It is noteworthy that in fat droplets nearly a half of the lycopene was in the *cis* configuration under all experimental protocols. The 1/1 ratio between *all-trans* and *cis* lycopene was proposed to represent the equilibrium under physiological conditions [2]. Fat droplets of hepatic stellate cells are not surrounded by a membrane [6, 7]. Import and export of different compounds are active processes that often involve enzymatic modifications of the transported compounds, such as esterification of retinol into retinyl esters when they are stored in fat droplets and their hydrolysis when exported [16]. In order to be enzymatically processed, retinol has to be presented to the corresponding enzymes associated with the cellular retinol-binding protein (CRBP). The fact that under present experimental conditions isomerisation of lycopene was saturable indicated that the process may be dependent both upon enzymes and carriers, which are at present unknown. It is also notable that the membrane fraction of lycopene, which is by far the major one, contained 30% of *cis* isomers. Since extracellular lycopene was exclusively in the *all-trans* form, the membrane loading of *cis* isoforms could only occur from the cytosolic side or during the intracellular turnover of cell membranes, in agreement with the relatively high *cis*-isomer content in cytoplasm.

It is also unexpected that lycopene alone was able to induce expression of the lipocyte phenotype in GRX cells. Up to now, two pathways of lipocyte induction in GRX myofibroblasts have been described. Both retinol and β-carotene produce retinoic acid, which is the classical inducer of lipocyte hyperplasia in vivo. Alternatively, indomethacin in combination with insulin can induce the lipocyte phenotype in GRX cells, acting directly on the PPARγ signaling pathway [6, 20, 25]. Lipocytes are considered to be resting hepatic stellate cells, while activated myofibroblasts are engaged in fibrogenesis and involved in hepatic fibrosis and cirrhosis [35]. In this context, lycopene-mediated induction of the lipocyte phenotype can have a protective role in hepatic pathologies leading to fibrosis. Simultaneously, enforced induction of the myofibroblast phenotype with loss of lipid droplets may decrease the stellate cell capacity to store lycopene. In a recent study of a schistosome-infected population, lycopene was the only serum carotenoid that suffered a threefold decrease in infected patients [13]. Schistosomal infection involves both hyperplasia of portal connective tissue cells and activation of hepatic stellate cells that are induced to express the myofibroblast phenotype [5, 11, 19]. The decreased capacity of lycopene handling may be one of the aspects of chronic hepatic diseases.

In conclusion, the present study has shown that hepatic stellate cells can uptake extracellular *all-trans* lycopene, which induces the expression of the lipocyte phenotype with formation of lipid droplets in which lycopene is to be stored. The lycopene accumulation in lipid droplets is associated with its isomerisation into at least two *cis* isoforms. This process is saturable and its biochemical mechanisms are at present unknown.

Acknowledgments We thank Adriana Mercadante and Itaciara Nunes for help in spectrum-UV analyses of lycopene isomer standards. This study was sponsored by CNPq grants of the Brazilian Ministry of Science and Technology and by FAPERJ grants of the Rio de Janeiro State Government.

References

1. Blaner WS, Olson JA (1994) Retinol and retinoic acid metabolism. In: Sporn MB, Roberts AB, Goodman S (eds) The retinoids: biology, chemistry and medicine, 2nd edn. Raven Press, New York, pp 229–255
2. Boileau AC, Merchen NR, Wasson K, Atkinson CA, Erdman JW Jr (1999) Cis-lycopene is more bioavailable than trans lycopene in vitro and also in vivo in the lymph cannulated ferret. J Nutr 129:1176–1181
3. Boileau TWM, Boileau AC, Erdman JW Jr (2002) Bioavailability of *all-trans* and *cis*-isomers of lycopene. Biol Med 227:914–919
4. Boileau TWM, Clinton SK, Zaripheh S, Monaco MH, Donovan SM, Erdman JW Jr (2001) Testosterone and food restriction modulate hepatic lycopene isomer concentrations in male F344 rats. J Nutr 131:1746–1752
5. Boloukhère M, Baldo-Correa E, Borojevic R (1993) Experimental schistosomiasis mansoni: characterization of connective tissue cells in hepatic periovular granulomas. J Submicrosc Cytol Pathol 25:505–517
6. Borojevic R, Guaragna RM, Margis R, Dutra HS (1990) In vitro induction of the fat-storing phenotype in a liver connective tissue cell line-GRX. In Vitro Cell Dev Biol 26:361–368
7. Borojevic R, Monteiro ANA, Vinhas SA, Domont GB, Mourão PAS, Emonard H, Grimaldi-Junior G, Grimaud JA (1985) Establishment of a continuous cell line from fibrotic schistosomal granulomas in mice livers. In Vitro Cell Dev Biol 21:382–390
8. Chalabi N, Delort L, Le Corre L, Satih S, Bignon YJ, Bernard-Gallon DJ (2006) Gene signature of breast cancer cell lines treated with lycopene. Pharmacogenomics 7:663–672
9. Chalabi N, Le Corre L, Maurizis JC, Bignon YJ, Bernard-Gallon DJ (2004) The effect of lycopene on the proliferation of human breast cells and BRCA1 and BRCA2 gene expression. Eur J Cancer 40:1768–1775
10. Chalabi N, Satih S, Delort L, Bignon YJ, Bernard-Gallon DJ (2007) Expression profiling by whole genome microarray hybridization reveals differential gene expression in breast cancer cell lines after lycopene exposure. Biochim Biophys Acta 1796:124–130
11. Chang D, Ramalho LN, Ramalho FS, Martinelli AL, Zucoloto S (2006) Hepatic stellate cells in human schistosomiasis: a comparative immunohistochemical study with liver cirrhosis. Acta Trop 97:318–323
12. Clinton SK (1998) Lycopene: chemistry, biology, and implications for human health and disease. Nutr Rev 56:35–51
13. Eboumbou C, Steghens JP, Abdallahi OM, Mirghani A, Gallian P, van Kappel A, Qurashi A, Gharib B, De Reggi M (2005) Circulating markers of oxidative stress and liver fibrosis in Sudanese subjects at risk of schistosomiasis and hepatits. Acta Trop 94:99–106
14. Ferreira AL, Yeum KJ, Liu C, Smith D, Krinsky NI, Wang XD, Russell RM (2000) Tissue distribution of lycopene in ferrets and rats after lycopene supplementation. J Nutr 130:1256–1260
15. Fortuna VA, Martucci RB, Trugo L, Borojevic R (2003) Hepatic stellate cells uptake of retinol associated with retinol-binding protein or with bovine serum albumin. J Cell Biochem 90:792–805
16. Fortuna VA, Trugo L, Borojevic R (2001) Acyl-CoA:retinol acyltransferase (ARAT) and lecithin:retinol acyltransferase (LRAT) activation during the lipocyte phenotype induction in hepatic stellate cells. J Nutr Biochem 12:610–621
17. Gajic M, Zaripheh S, Sun F, Erdman JW Jr (2006) Apo-8′-lycopenal and apo-12′-lycopenal are metabolic products of lycopene in rat liver. J Nutr 136:1552–1557
18. Gann PH, Ma J, Giovannucci E, Willett W, Sacks FM, Hennekens CH, Stampfer MJ (1999) Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. Cancer Res 59:1225–1230
19. Grimaud JA, Borojevic R (1986) Portal fibrosis: intrahepatic portal vein pathology in chronic human schistosomiasis mansoni. J Submicrosc Cytol 18:783–793
20. Guimarães EL, Franceschi MF, Andrade CM, Guaragna RM, Borojevic R, Margis R, Bernard EA, Guma FC (2007) Hepatic stellate cell line modulates lipogenic transcription factors. Liver Int 27:1255–1264
21. Guma FCR, Mello TG, Mermelstein CS, Fortuna VA, Wofchuk ST, Gottfried C, Guaragna RM, Costa ML, Borojevic R (2001) Intermediate filaments modulation in an in vitro model of the hepatic stellate cell activation or conversion into the lipocyte phenotype. Biochem Cell Biol 79:409–417
22. Harrison EH, Blaner WS, Goodman DS, Ross AC (1987) Subcellular localization of retinoids, RBP and ARAT in rat liver. J Lipid Res 28:973–981

23. Herrmann J, Gressner AM, Weiskirchen R (2007) Immortal hepatic stellate cell lines: useful tools to study heaptic stellate cell biology and function? J Cell Mol Med 11:704–722
24. Huang CS, Shih MK, Chuang CH, Hu ML (2005) Lycopene inhibits cell migration and invasion and upregulates Nm23-H1 in a highly invasive hepatocarcinoma, SK-Hep-1 cells. J Nutr 135:2119–2123
25. Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA (1997) Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem 272:3406–3410
26. Lindshield BL, King JL, Wyss A, Goralczyk R, Lu CH, Ford NA, Erdman JW Jr (2008) Lycopene biodistribution is altered in 15,15′-carotenoid monooxygenase knockout mice. J Nutr 12:2367–2371
27. Liu C, Russell RM, Wang XD (2006) Lycopene supplementation prevents smoke-induced changes in p53, p53 phosphorylation, cell proliferation, and apoptosis in the gastric mucosa of ferrets. J Nutr 136:106–111
28. Margis R, Borojevic R (1989) Retinoid-mediated Induction of the fat-storing phenotype in a liver connective tissue cell line (GRX). Biochim Biophys Acta 1011:1–5
29. Martucci RB, Ziulkoski AL, Fortuna VA, Guaragna RM, Guma FC, Trugo LC, Borojevic R (2004) B-Carotene storage, conversion to retinoic acid, and induction of the lipocyte phenotype in hepatic stellate cells. J Cell Biochem 92:414–423
30. McClain M, Bausch J (2003) Summary of safety studies conducted with synthetic lycopene. Regul Toxicol Pharmacol 37:274–285
31. Moraru C, Lee TC (2005) Kinetic studies of lycopene isomerisation in a tributyrin model system at gastric pH. J Agric Food Chem 53:8997–9004
32. Nahum A, Zeller L, Danilenko M, Prall OW, Watts CK, Sutherland RL, Levy J, Sharoni Y (2006) Lycopene inhibition of IGF-induced cancer cell growth depends on the level of cyclin D1. Eur J Nutr 45:275–282
33. Nunes I, Mercadante AZ (2004) Production of lycopene crystals from tomato waste. Food Sci Technol 24:440–447
34. Russell RM (1998) Physiological and clinical significance of carotenoids. Int J Vitam Nutr Res 68:349–353
35. Sato M, Suzuki S, Senoo H (2003) Hepatic stellate cells: unique characteristics in cell biology and phenotype. Cell Struct Funct 28:105–112
36. Shahrzad S, Cadenas E, Sevanian A, Packer L (2002) Impact of water-dispersible beadlets as a vehicle for the delivery of carotenoids to cultured cells. Biofactors 16:83–91
37. Smith PK et al (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150(1):76–85
38. Stahl W, Schwarz W, Sundquist AR, Sies H (1992) Cis-trans isomers of lycopene and beta-carotene in human serum and tissues. Arch Biochem Biophys 294:173–177
39. Vertzoni MV, Reppas C, Archontaki HA (2005) Optimized determination of lycopene in canine plasma using reversed-phase high-performance liquid chromatography. J Chromatogr B 819: 149–154
40. Vicente CP, Fortuna VA, Margis R, Trugo LC, Borojevic R (1998) Retinol uptake and metabolism, and cellular retinol binding protein in an in vitro model of hepatic stellate cells. Mol Cell Biochem 187:11–21
41. Wamer WG, Wei RR, Matusik JE, Kornhauser A, Dunkel VC (1993) β-Carotene uptake, metabolism and distribution in BALB/c 3T3 cells. Nutr Cancer 19:31–41
42. Yeum KJ, Booth SL, Sadowski JA, Liu C, Tang G, Krinsky NI, Russell RM (1996) Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. Am J Clin Nutr 64:594–602
43. Zaripheh S, Boileau TWM, Lila MA, Erdman JW Jr (2003) [^{14}C]-Lycopene and [^{14}C]-labeled polar products are differentially distributed in tissues of F344 rats prefed Lycopene. J Nutr 133:4189–4195