Absorption, storage, and distribution of beta-carotene in normal and beta-carotene-fed rats: roles of parenchymal and stellate cells

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Abstract Absorption and storage of [^{14}C]beta-carotene in control and beta-carotene-fed (BC-fed) rats were determined. Prefeeding with beta-carotene for 2 weeks caused a 1.9-fold stimulation of its own absorption as well as its conversion to retinyl esters, whereas the absorption of [^{3}H]retinyl acetate was unaffected. The liver and the lungs accounted for 60% and 30%, respectively, of the total recovered ^{14}C radioactivity in both control and BC-fed groups. Beta-carotene accounted for 80–87% of the recovered ^{14}C radioactivity in both the liver and the lung. Subcellular distribution of [^{14}C]beta-carotene in both control and BC-fed groups revealed that the cytosol was the major fraction accounting for 44.4% and 26.8% of the radioactivity in the liver and lungs, respectively. Distribution of beta-carotene among liver parenchymal (PC) and stellate cells (STC) was determined in the two groups. Based on radioactivity, the PC and STC contained 22% and 78% of the total, respectively, in the control group; the corresponding values for the PC and STC in the BC-fed group were 48% and 52% of the total radioactivity, respectively. Based on the beta-carotene concentration following chronic beta-carotene feeding, PC contained 75.5% while the STC had 24.5% of the total beta-carotene. Thus, parenchymal cells seem to be the major hepatic storage site for dietary beta-carotene after chronic feeding. —**Lakshman, M. R., K. A. Asher, M. G. Attlesey, S. Satchithanandam, I. Mychkovsky, and P. J. Coutlakis.** Absorption, storage, and distribution of beta-carotene in normal and beta-carotene-fed rats: roles of parenchymal and stellate cells. *J. Lipid Res.* 1989. **30:** 1545–1550.

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Beta-carotene, a dietary precursor of vitamin A, has gained considerable attention as a possible antitumor agent (1). Epidemiological studies have indicated that populations with low serum vitamin A have a higher incidence of cancer than those with normal or higher serum vitamin A levels (2). However, in view of the toxicity of prophylactic doses of vitamin A, there has been an intensive search for alternative sources of vitamin A-like compounds with antitumor properties with negligible toxicity. An inverse correlation has been established between beta-carotene consumption and the incidence of cancer (2,3). Furthermore, Mathews-Roth (4) showed 4,4'-diketo beta-carotene, which has no provitamin A activity, can prevent UV-induced skin tumors in hairless mice. Thus, carotenoids may exert their antitumor property per se and not after conversion to retinoids. In view of the fact that beta-carotene has negligible toxicity, it may prove to be a viable mode of chemoprevention of carcinogenesis. Therefore it is important to test whether beta-carotene can be absorbed and stored without any biotransformation so that it can be of significant value in preventing carcinogenesis. It would also be important to answer the following questions: *a*) how can the absorption of beta-carotene as such be increased; *b*) which tissue(s) store beta-carotene; *c*) in which subcellular fraction of the tissue is beta-carotene localized; and finally, *d*) what are the relative roles of parenchymal and stellate cells in the hepatic storage of beta-carotene?

Rodents are very efficient in converting beta-carotene to vitamin A (5). Therefore very little of the ingested beta-carotene is absorbed as such unless the carotene is fed in significant quantities (6,7). Thus, the elegant studies of Shapiro, Mott, and Machlin (7) showed that there was a dose-dependent accumulation of intact beta-carotene in rat tissues, the liver being the major storage site. The liver is also the major storage site for retinyl esters plus retinol with stellate cells being the primary site of its accumulation with widely varying dosages of retinyl acetate (8–12).

Abbreviations: BC, beta-carotene; PC, parenchymal cells; STC, stellate cells; HPLC, high performance liquid chromatography.

EXPERIMENTAL

Animals and diets

Male Wistar-Furth rats (body weight ~250 g) were procured from Charles River, Wilmington, MA. All the animals were maintained on the normal rat chow (Wayne Lablox, Allied Mills, Inc., Chicago, IL) for 2 weeks before experimentation. The rats were divided into two groups. The BC-fed group was maintained for 2 weeks ad libitum on the normal powdered rat chow (Wayne Lablox, Allied Mills, Inc., Chicago, IL) containing 0.2% (w/w) beta-carotene in the form of beadlets (Hoffman-La Roche, Nutley, NJ; beta-carotene beadlets are reported to be composed of beta-carotene compounded with gelatin, sucrose, food starch, and peanut oil. Ascorbyl palmitate and *dl*-alpha tocopherol are added as antioxidants. The respective concentrations of all the above components except beta-carotene are not listed by the company. The reported concentration of beta-carotene is 10% (w/w) in the beadlets). The control group was fed the same diet containing the placebo beadlets without the beta-carotene. All experimental protocols were performed using these two groups of rats which will be hereafter referred to as BC-fed and control groups, respectively.

Safety precautions

All the procedures described below were carried out in a safety yellow light room. All carotenoid and retinoid compounds, tissues and extracts were stored at −80°C under an atmosphere of nitrogen.

Intestinal metabolism of carotenoids in vivo: effect of pre-feeding beta-carotene upon the absorption and fate of intragastrically administered [^{14}C]beta-carotene

The thoracic duct and the stomach of each rat from BC-fed and control groups (eight per group) were cannulated. A single dose of 0.2 ml corn oil containing [15,15'-^{14}C]beta-carotene (370 nmol and 1 μCi) and [11,12-^{3}H]retinyl acetate (67 nmol and 20 μCi) was infused intragastrically over a 1-h period. Lymph was collected on ice over the next 24 h and lyophilized. The residue was extracted according to Folch et al. (13). The lipid extract was analyzed for both radioactivity and beta-carotene and retinoids as described below.

Preparation of beta-carotene-labeled lymph chylomicrons

The thoracic lymph duct was cannulated (14) in three normal rats intragastrically fed 1 ml corn oil/rat, and the lymph was collected on ice over the next 16 h. Chylomicrons were isolated from the collected lymph by flotation for $3 \times 10^6 g$-av_{min}. [15,15'-^{14}C]Beta-carotene (1 μCi and 0.6 μmol) in 1 ml methylene chloride was mixed with 0.66 ml of dimethylsulfoxide and the methylene chloride was evaporated under a gentle stream of nitrogen. This solution of dimethylsulfoxide containing the labeled carotene was injected as a jet stream with the aid of a Hamilton syringe into 1 ml of 0.154 M NaCl–0.25 mM EDTA (saline-EDTA) solution with vigorous stirring. The resulting solution containing the fine suspension of beta-carotene was incubated with 2 ml of rat lymph chylomicrons (2 mg cholesterol/ml) at 37°C for 2 h in the dark. The reaction mixture was then extensively dialyzed against saline–EDTA. Each ml of the final chylomicron solution contained 3.6×10^5 dpm and 100 nmol of beta-carotene. The average efficiency of labeled beta-carotene incorporation into lymph chylomicrons according to this procedure was 60%.

Extra-intestinal metabolism of carotenoids: fate of chylomicron-bound beta-carotene after intravenous adminstration

The jugular vein of each rat from BC-fed and control groups (five per group) was cannulated and 1 ml of lymph chylomicrons containing labeled beta-carotene (3.6×10^5 dpm and 100 nmol) was infused intrajugularly over a 1-h period. The rats were killed after 16 h by aortic exsanguination, and the plasma, liver, adipose tissue, kidneys, and lungs were isolated. The lipids were extracted (13) from each organ (1 ml lyophilized plasma, 4 g each of liver and adipose tissue, 1 g each of lungs and kidneys). The lipid extracts of these organs were analyzed for carotenoids and retinoids.

Saponification and alumina chromatography

Whenever saponification was necessary, the lipid extract of each tissue was saponified for 30 min at 60°C in 1 N ethanolic KOH containing 5% (w/w) pyrogallol and 0.005% (w/v) butylated hydroxy-toluene (BHT), after which the reaction mixture was twice extracted with 20-ml portions of light petroleum. Under these conditions the loss of added beta-carotene or retinol was less than 5%. Whenever beta-carotene and retinol had to be separated, the saponified lipid extract was subjected to alumina chromatography on 10 g of a 1 × 12 cm column of 5% (v/w) water-deactivated neutral alumina (Sigma Chemical Co, St. Louis MO). Beta-carotene was completely eluted by 2% acetone in light petroleum (v/v) while retinol was eluted by 10% acetone in light petroleum. The recoveries of added beta-carotene and retinol throughout this chromatography procedure were 80–85%.

Subcellular distribution of intravenously administered beta-carotene in the liver

The procedure was essentially that of Mayne and Parker (15). A portion of each liver was homogenized with 9 volumes of STE buffer. The homogenate was centrifuged at 700 *g* for 10 min. The postnuclear supernatant solution

was centrifuged at 15,000 *g* for 5.5 min yielding the mitochondrial pellet which was washed by resuspension in the original buffer followed by recentrifugation at 15,000 *g* for 5.5 min. The combined postmitochondrial supernatant solution was centrifuged at 25,000 *g* for 10 min to yield the lysosomal pellet which was also washed and pelleted again by recentrifugation. The combined post-lysosomal supernatant solution was finally centrifuged at 105,000 *g* for 60 min to yield the microsomal pellet and the cytosol fractions. The fat layer floating on top of the cytosol fraction was also included with the cytosol fraction. Each subcellular fraction except the cytosol fraction was resuspended in a small volume of the original buffer and an aliquot was analyzed for radioactivity.

Distribution of absorbed beta-carotene among liver parenchymal (PC) and stellate cells (STC)

The jugular vein of each rat from the BC-fed and the control groups was cannulated and 1 ml of lymph chylomicrons containing labeled beta-carotene (3.6×10^5 dpm and 100 nmol) was infused intrajugularly over a 1-h period. After 16 h each rat was set up for liver perfusion for the isolation of crude hepatocytes (8,11). Just before the liver was perfused with collagenase, the smallest lobe of the liver was dissected out after ligating its vascular connection. This lobe was weighed and stored at −80°C until analyzed for total beta-carotene and retinoids. The purified PC were isolated from the crude hepatocytes by centrifugal elutriation (11). The PC preparation was more than 99% pure by this procedure and was virtually devoid of STC. The purified STC were isolated from approximately 150×10^6 crude hepatocytes by pronase (Merck) digestion followed by Metrizamide gradient centrifugation (8). The STC preparation was around 80% pure but was virtually free of PC. The only contaminating cells were the endothelial cells which are known to carry only negligible amounts of retinoids (10). The purity of each of the PC and STC preparations was verified by counting the STC by fluorescence microscopy. The lipid extract (13) of each liver lobe and of each cell fraction was analyzed for radioactivity and for carotenoids and retinoids by HPLC as described below. Based on beta-carotene determinations in PC and STC fractions, the values/g wet wt of liver for these cell fractions were calculated assuming 108×10^6 PC and 16.2×10^6 STC are equivalent to 1 g wet wt of liver (10). Thus, all the values are expressed per g total liver.

Analysis of radioactivity

All radioactive measurements were carried out in a Beckman Model LS9800 liquid scintillation spectrometer with automatic quench compensation.

HPLC analysis of beta-carotene and retinoids

All organic solvents were of HPLC grade and had 0.005% BHT (w/v) whenever used for lipid extraction. All tissues or cell fractions were extracted for lipids (13), filtered through a 0.45 μm glass-fiber filter, and evaporated with a gentle stream of nitrogen; finally, the lipid extract was redissolved in methylene chloride. HPLC analyses were carried out using a Gilson HPLC system equipped with a Kratos Model 783 variable wavelength detector. An aliquot of the lipid extract in methylene chloride was subjected to HPLC on an Axxiom, Axxi-Chrom C-18 (ODS-3 μm) column (0.46 × 10 cm; Thomson Instruments & Co, Springfield, Va) using an isocratic system of 100% methanol containing 0.5% (w/v) ammonium acetate at an initial flow rate of 1 ml/min for 3 min after which the solvent flow rate was increased to 1.5 ml/min. Under these conditions, authentic retinol, retinyl palmitate, and beta-carotene had retention times of 2.5, 11.2, and 16.2 min, respectively. Recoveries (through the extraction and HPLC procedures described above) of known quantities of beta-carotene and retinyl palmitate added to the tissues were more than 95% while the recovery of added retinol was 92 ± 5% (average of five independent analyses).

RESULTS AND DISCUSSION

It is clear from **Table 1** that pre-feeding the animal with beta-carotene caused a 1.9-fold stimulation of its own absorption, whereas the absorption of a closely related compound, retinyl acetate, was unaffected. Furthermore, based

TABLE 1. Effect of prefeeding beta-carotene on absorption of [^{14}C]beta-carotene and [11,12-^{3}H]retinyl acetate

	Lymph Radioactivity	
Pretreatment	^{3}H	^{14}C[a]
	% of the administered dose	
Control	16.1 ± 3.9	3.6 ± 0.9
Beta-carotene	19.4 ± 6.1[b]	6.8 ± 2.1[c]

Two groups of rats (eight per group) were maintained on the normal rat chow diet ad lib for 2 weeks. During this period, one group received, by stomach tube, a daily dose of beta-carotene (7.5 μmol in 0.5 ml corn oil) while the second group received the corn oil vehicle only. After 2 weeks, the thoracic duct and the stomach of each rat were cannulated. A single dose of 0.2 ml corn oil containing [15,15'-^{14}C]beta-carotene (370 nmol and 1 μCi) and [11,12-^{3}H]retinyl acetate (67 nmol and 20 μCi) was infused intragastrically over a 1-h period. Lymph was collected on ice over the next 24 h and the lipid extract was analyzed for both radioactivity and carotenoids/retinoids. Each value is the mean ± SD.

[a]Out of the ^{14}C radioactivity, 20.5% was recovered in beta-carotene and 79.5% in retinyl ester + retinol in both control and BC-fed groups.

[b]Not significant compared to the control group.

[c]Significantly increased by 189% ($P < 0.01$) compared to the control group.

on alumina chromatographic analysis of the saponified extract, 20% of the ^{14}Cradioactivity was recovered as beta-carotene while 80% was associated with the retinol regardless of whether the animals were from the control or the BC-fed group. These results have two implications. First, beta-carotene feeding leads to a 2-fold increase in the conversion of beta-carotene to retinyl ester plus retinol. This would imply that beta-carotene cleavage enzyme activity may be inducible by pre-feeding with beta-carotene. Second, prior saturation of the beta-carotene cleavage system with beta-carotene leads to a significant 2-fold increase in the absorption of the newly ingested carotene as such without being converted to retinal or other retinoid derivatives. The second implication may have important clinical significance regarding the possible anticarcinogenic role of beta-carotene and other carotenoids that escape intestinal cleavage.

The results on the fate of the absorbed beta-carotene are presented in **Table 2**. It must be pointed out that since beta-carotene (bound to chylomicrons) was infused intravenously in this experiment, unlike the experiment described in Table 1, beta-carotene had escaped the intestinal cleavage. Thus, the results presented in Table 2 reflect only the fate of beta-carotene after it had entered the systemic circulation. It is clear that the liver and lungs accounted for 34.8% and 16.6%, respectively, of the administered dose in the control group while the corresponding values for these two organs in the BC-fed group were 30.9% and 17.1%, respectively. However, none of the values in the BC-fed group was significantly different from the corresponding value in the control group ($P > 0.2$). The results imply that chronic beta-carotene feeding does not seem to affect the fate of intravenously administered beta-carotene in various tissues. The plasma, adipose tissue, and the kidneys had 2.6%, 1.2%, and 0.4% of the administered dose, respectively. Thus, the liver and the lung tissues together accounted for 90% of the radioactivity recovered in various tissues regardless of whether the animals were from the normal or the BC-fed group. Therefore, only the data for the recoveries in liver and lung tissues of both normal and BC-fed rats are presented in the table.

Further analysis of the saponified lipid extract of various organs showed that 80–87% of the radioactivity was due to beta-carotene, while the rest of that label was due to retinyl ester. This was in contrast to the recovery of beta-carotene and retinyl ester in the lymph after intraduodenal administration of beta-carotene (Table 1). Since a substantial percentage of the intravenously administered beta-carotene radioactivity remained as such in the liver and lungs, further metabolic fate and function of this carotene would be of importance. It is particularly intriguing to find such a large percentage of administered carotene radioactivity localized in the lung under these experimental conditions. To what extent this radioactivity is associated with the reticuloendothelial cells of the lung tissue remains to be clarified. However, as demonstrated below (see Table 4), almost all of the beta-carotene radioactivity in the liver is associated only with the parenchymal and stellate cells, not with the reticuloendothelial cells of the liver.

The possibility exists that the observed distribution of beta-carotene radioactivity among various tissues simply reflects the fate of administered chylomicrons rather than beta-carotene per se. However, such as possibility is unlikely in view of a similar distribution pattern of beta-carotene among various tissues after chronic feeding of beta-carotene to rats as observed by Shapiro et al (7). They (7) also reported that the mouse stores 10 times more beta-carotene in the lung than the Sprague-Dawley rat. These authors also failed to find significant amounts of beta-carotene in the adipose tissue after beta-carotene feeding as observed in the present study. Thus, unlike humans (16), the rat does not seem to store any measurable amount of beta-carotene in the adipose tissue even after chronic beta-carotene feeding.

Subcellular distribution of radioactivity in the liver from intravenously administered [^{14}C]beta-carotene (**Table 3**) revealed that 44.4% of the cellular radioactivity

TABLE 2. Recovery of beta-carotene and retinoids in liver and lungs after intravenous administration of [^{14}C]beta-carotene

	Radioactivity Recovered in			
	Liver		Lungs	
	Control	BC-Fed	Control	BC-Fed
% of Administered dose	34.8 ± 7.5	30.9 ± 3.8	16.6 ± 4.5	17.1 ± 1.9
% of Recovered dose	61.0 ± 2.1	57.9 ± 3.9	28.9 ± 2.5	32.3 ± 3.7
% Recovered as BC	82.4 ± 5.1	82.1 ± 5.0	80.4 ± 1.0	87.3 ± 1.8
% Recovered as retinoids	17.6 ± 2.1	17.9 ± 2.0	19.6 ± 1.0	12.7 ± 1.8

One ml of lymph chylomicrons containing labeled beta-carotene (3.6×10^5 dpm and 100 nmol) was infused intrajugularly over a 1-h period to control and BC-fed rats (five per group). The rats were killed after 16 h by aortic exsanguination and the lipid extracts of various organs were analyzed for beta-carotene (BC) and retinoids as described in the Experimental section. The recoveries in the plasma, kidneys, and the adipose tissues combined together amounted to a mean value of only 9.7 ± 1.5% of the total recovered dose in all organs. Each value is the mean ± SD.

TABLE 3. Subcellular distribution of radioactivity after intravenous administration of [^{14}C]beta-carotene

Subcellular Fraction	% of the Total Radioactivity Recovered	
	Liver	Lung
Nuclear + cell debris	19.4 ± 2.3	63.5 ± 12.6
Mitochondria	17.7 ± 3.1	5.5 ± 0.7
Lysosomes	11.6 ± 3.3	3.9 ± 1.0
Microsomes	7.0 ± 2.5	0.4 ± 0.1
Cytosol	44.4 ± 1.1	26.8 ± 4.6

One ml lymph chylomicrons containing labeled beta-carotene (3.6×10^5 dpm and 100 nmol) was infused intrajugularly over a 1-h period to four animals from the BC-fed group. The rats were killed after 16 h by aortic exsanguination and the liver and the lungs were isolated. Subcellular fractions of the respective homogenates were isolated as described in the Experimental section. An aliquot of each fraction was analyzed for radioactivity. Each value is the mean ± SD.

was recovered in the cytosol fraction. The nuclear and mitochondrial fractions accounted for 19.4% and 17.7%, respectively and the lysosomal and microsomal fractions had only 11.6% and 7%, respectively. It is noteworthy that the subcellular distribution pattern based on ^{14}C radioactivity (Table 3) agrees reasonably well with those of Mayne and Parker (15) in chicken liver based on the absolute concentration of beta-carotene except that data were not reported for the cytosol fraction in that study. Thus, the observed radioactivities in various subcellular fractions substantially reflect their absolute concentrations of beta-carotene. This is probably because 82% of the recovered radioactivity was found in the beta-carotene fraction in the whole liver. Whether or not each subcellular fraction exhibits a similar percentage distribution of beta-carotene as the whole liver remains to be seen. However, it should be pointed out that other metabolites of beta-carotene metabolism, particularly retinoic acid, may also be present particularly in the cytosol fraction, albeit in low concentration. The formation of retinoic acid from beta-carotene in vitro by cytosol preparations from rat liver, intestine, kidney, lung, and testes has recently been reported (17).

The lung tissue also exhibited a similar pattern of radioactivity distribution among the subcellular components as the liver. The only exception was the nuclear + cell debris fraction which had 63.5% of the radioactivity in the case of lung tissue, whereas only 19.5% was associated with this fraction in the liver. Attempts are being made to determine how much of the radioactivity in this fraction is due to the cell debris sedimenting with the nuclear fraction in the case of lung homogenate.

The distribution of beta-carotene in various liver cells after intravenous administration of labeled beta-carotene bound to chylomicrons is depicted in **Table 4**. Based on radioactivity, the PC and STC had 22% and 78% of the total liver beta-carotene radioactivity, respectively, in the control animals, whereas the corresponding values in the two cell types in BC-fed animals were 48% and 52%, respectively. The apparent discrepancy between the data from control and BC-fed groups implies that the capacity of STC to store beta-carotene may be lower than that of parenchymal cells. That is why the stellate cells contain more of the beta-carotene radioactivity in non-BC-fed animals, whereas parenchymal cells accumulate the label in BC-fed rats. This is further confirmed by the observed distribution of beta-carotene concentration among PC and STC in BC-fed rats (Table 4). PC was found to have 75.5% of the total beta-carotene while the STC had the remaining. Thus, the PC seem to be the major cell type of the liver accumulating beta-carotene in BC-fed rats. This was in sharp contrast to the storage of retinyl palmitate which was localized predominantly in the STC fraction of the liver (8–12). It is pertinent to point out that the results of the distribution of retinyl ester plus retinol among parenchymal and stellate cells were similar to already published results (8–12) and, therefore, are not reported again. Thus, PC seem to have a greater capacity than STC for the hepatic storage of dietary beta-carotene while STC are the main storage site for retinyl ester plus retinol. This implies that PC may be more important than STC in the further metabolism of beta-carotene in the liver. This is not surprising in view of the known role of parenchymal cells in the apoE receptor-mediated uptake and metabolism of chylomicron remnants (18–21).

TABLE 4. Hepatic distribution of beta-carotene among parenchymal and stellate cells in control and beta-carotene-fed rats

Beta-Carotene Recovered	Total Liver		Parenchymal Cells		Stellate Cells	
	Control	BC-Fed	Control	BC-Fed	Control	BC-Fed
dpm × 10^{-4}/g wet wt	1.08 ± 0.13	1.09 ± 0.14	0.24 ± 0.04	0.52 ± 0.12	0.83 ± 0.12	0.57 ± 0.1
As % of total liver	100	100	22 ± 2	48 ± 6	78 ± 3	52 ± 6
nmol/g wet wt		37.4 ± 5.1		28.5 ± 6.4		9 ± 2.4
As % of total liver		100		75.5 ± 8.5		24.5 ± 8.5

One ml lymph chylomicrons containing labeled beta-carotene (3.6×10^5 dpm and 100 nmol) was infused intrajugularly over a 1-h period to four animals from the control group and six animals from the BC-fed group. After 16 h, hepatic parenchymal and stellate cells were isolated from each animal and each cell fraction was analyzed for radioactivity and beta-carotene as described in the Experimental section. Each value is the mean ± SD.

It is pertinent to note that rat plasma has predominantly high density lipoprotein and very little low density lipoprotein (LDL), unlike human plasma. Therefore, the distribution pattern of beta-carotene among various tissues observed in the present study may be altered in humans if receptor-mediated LDL uptake is the major mechanism of tissue uptake of beta-carotene (22,23).

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