Intestinal absorption and metabolism of 9-*cis*-β-carotene in vivo: biosynthesis of 9-*cis*-retinoic acid

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Abstract This study was done to examine the intestinal absorption and cleavage of 9-*cis*-β-carotene in vivo. A micellar solution, containing either no addition or 10 μmol of 9-*cis*- or all-*trans*-β-carotene, was perfused for 2 h through the upper portion of the small intestine of ferrets. The effluent of a mesenteric lymph duct cannulation was collected, as well as intestinal mucosa scrapings, a portal blood sample, and a liver biopsy, both before and after perfusion. Carotenoids and retinoids were measured by reverse-phase, high performance liquid chromatography. 9-*Cis*- and all-*trans*-β-carotene were transported equally well into mesenteric lymph, although the intestinal concentration of the corresponding isomer was tenfold higher after perfusion of the 9-*cis*- isomer than after perfusion of all-*trans*-β-carotene. Regardless of which isomer was used, perfusion of β-carotene resulted in the biosynthesis of similar amounts of retinoic acid in portal blood, liver, and intestine. However, after the perfusion of all-*trans*-β-carotene, all the retinoic acid formed was in the all-*trans*- form, whereas the perfusion of 9-*cis*-β-carotene resulted in the biosynthesis of about 50% of the total retinoic acid as the 9-*cis*- isomer. We conclude that in the in vivo ferret model, 9-*cis*-β-carotene has a good bioavailability and is a precursor of 9-*cis*-retinoic acid.—**Hébuterne, X., X-D. Wang, E. J. Johnson, N. L. Krinsky, and R. M. Russell.** Intestinal absorption and metabolism of 9-*cis*-β-carotene in vivo: biosynthesis of 9-*cis*-retinoic acid. *J. Lipid Res.* 1995. **36:** 1264–1273.

Supplementary key words all-*trans*-β-carotene • all-*trans*-retinoic acid • vitamin A • β-carotene cleavage • HPLC • ferret

Since 1981, it has been proposed that β-carotene (β-C) may reduce the risk of certain types of cancer (1). Both epidemiologic (2) and biologic studies (3) support this hypothesis, and it generally has been accepted that persons with a high intake of dietary β-C and/or high serum levels of β-C have a lower risk of cancer, especially lung cancer. However, the first randomized trial of supplementation with 20 mg/day synthetic all-*trans*-β-C in a large population of smokers found no reduction in the incidence of lung cancer (4).

The mechanism of action of β-C in protecting against cancer is not established. One possible mechanism is attributable to the antioxidant and free radical trapping properties of the intact β-C molecule (5). However, the effects of β-C may also be attributed to one of its metabolites, retinoic acid (RA) which is known to induce cell differentiation (6) and which has been successfully used in the treatment of promyelocytic leukemia (7, 8). Moreover, the anticarcinogenic effect of β-C may differ with a different isomeric form of the molecule.

Both *trans*- and *cis*- isomers of β-C are present in food (9, 10), and biostereo-isomerization of all-*trans*-carotenoids has been observed in the body (11, 12). Several stereoisomers of carotenoids (13), and particularly of β-C (14), have been found to be present in human blood, and 9-*cis*-β-carotene may account for up to 25% of the total β-C in human liver (15). Little is known of the intestinal absorption of various β-C isomers. It has been shown that after supplementation of equal amounts of 9-*cis*- and all-*trans*-β-C, only all-*trans*-β-C produced a rise in human serum (16–18), suggesting that the 9-*cis*- form of the molecule is not well absorbed. However, in chicks and rats, supplementation with 9-*cis*-β-C resulted in a large accumulation of this isomer in the liver (19, 20) suggesting good absorption and possibly a more rapid tissue uptake of the 9-*cis*- isomer.

Stereoisomers of retinoic acid may also be involved in the anticarcinogenic properties of β-C. The occurrence of unidentified isomers of RA as well as 9-*cis*- and 13-*cis*-RA in human blood and tissues has been reported (21–25). Two retinoid classes of nuclear receptors (i.e., RAR and RXR) have distinct physiologic properties. All-*trans*-RA binds the RAR but not the RXR. The recent identification of 9-*cis*-RA as the specific ligand for the RXR (25–27) raises questions about the source of 9-*cis*-RA in the body.

Abbreviations: β-C, β-carotene; RA, retinoic acid; HPLC, high performance liquid chromatography; HNRC, Human Nutrition Research Center on Aging.

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The present study was done to elucidate the intestinal absorption and metabolism of 9-*cis*-β-C in vivo, compared to the all-*trans*- form of the molecule. β-C was administered via intestinal perfusion in the ferret, which has been demonstrated to be a suitable model because, like the human, the ferret is able to absorb intact β-C in lymph (28) and to convert β-C to RA (29).

MATERIALS AND METHODS

Chemical products

All-*trans*-RA, retinyl acetate, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), Krebs phosphate buffer, α-tocopherol, oleic acid, sodium taurocholate, and other chemicals were purchased from Sigma Co. (St. Louis, MO). All-*trans*-β-C, 13-*cis*-β-C, 9-*cis*-β-C, 9-*cis*-RA, 13-*cis*-RA, all-*trans*-9-(4-methoxy-2,3,6-trimethyl phenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid (TMMP), and echinenone were generous gifts from Hoffmann-La Roche Inc., Nutley, NJ. All carotenoids and retinoids were stored under N_2 at −70°C. All solutions were prepared under red light immediately before use. β-Carotene was purified by chromatography on a 5% water-weakened alumina column. The purity of β-C achieved was >98%. All high performance liquid chromatography (HPLC) solvents were obtained from Baker Chemical Co. (Philipsburg, NJ) and were filtered through a 0.45 μm membrane filter before use.

Animals

Male ferrets (*Mustela putorius furo*), from Marshall Farms (North Rose, NY) were housed in an American Association of Accreditation of Laboratory Care-accredited animal facility at the Human Nutrition Research Center on Aging (HNRC) at Tufts University. They were fed dry ferret food (Bil. Jac®, Win-Hy Foods, Tulsa, OK) and water ad libitum. The dry ferret food contained β-C 0.54 μg/g and retinol 17.7 μg/g, as determined by HPLC in our laboratory. Surgical procedures in the experiments were approved by the Animal Care Committee at Tufts University and surgery was conducted under aseptic conditions in the Division of Comparative Medicine at the HNRC.

Study design

We used procedures previously described (28) with certain modifications. Briefly, after an overnight fast, 3.0 mL corn oil was administered orally to ferrets to dilate the intestinal lymphatics. Thirty minutes later, ketamine hydrochloride (35 mg/kg) and xylazine (3 mg/kg) were administered intramuscularly to induce anesthesia. Ferrets were intubated with 3.0 mm ID endotracheal tubes, and anesthesia was maintained with 2–3% isoflurane in 100% oxygen. Anesthetized ferrets were kept on circulating hot water blankets at 38°C. Through a midline abdominal incision, the mesenteric lymph duct was cannulated using a polyethylene catheter (1.27 mm OD, 0.86 mm ID; PE-90, Clay Adams, Becton Dickinson, Parsippany, NJ). The portal vein was cannulated using a heparinized polyethylene catheter (1.22 mm OD, 0.76 mm ID; PE-60, Clay Adams). Both lymph and portal tubes were secured with surgical glue. A proximal inflow catheter (0.64 cm OD, 0.32 cm ID; Tygon flexing plastic tubing, Norton, Akron, OH) was inserted into the jejunum 5 cm distally to the ligament of Treitz, the proximal outflow catheter was introduced 50 cm distally. To prevent the perfusate from washing back into the stomach or continuing into the intestine, catheters were secured by encircling ligatures. The intestinal segment was flushed with normal saline to remove intestinal contents.

A micellar solution containing 0.5 μM α-tocopherol, 2.5 mM oleic acid, and 10 mM sodium taurocholate in Krebs phosphate buffer at pH 7.0 was formed by sonication for 15 min at 80 W of power (Branson, Shelton, CT). All the animals received first this micellar solution for 60 min at 2.0 mL/min. In Group 1 (control group), three animals received the micellar solution without any carotenoids or retinoids during 2 h at a flow rate of 2 mL/min. In Group 2, four animals received the micellar solution plus 10 μM of 9-*cis*-β-C at a flow rate of 2 mL/min. In Group 3, four animals received the micellar solution plus 10 μM of all-*trans*-β-C at a flow rate of 2 mL/min. In groups 2 and 3, the β-C was added into the micellar solution immediately before the perfusion and was incorporated into the micelles by sonication (15 min at 80 W of power). The extent of micellar incorporation of β-C in the perfusate was examined by filtration through a 0.2 μm filter (Uniflotm, Schleicher & Schuell, Keene, NH) as suggested by Hollander, Dadufalza, and Sletten (30). The stability of β-C after sonication and after 8 h storage at room temperature was checked by HPLC. No oxidative products were detected and the purity of β-C (*cis*- or *trans*-form) was similar to the original β-C (>98%).

In all experiments, the lymph drainage was collected by gravity in 30-min collections. The portal vein cannula was sampled immediately before and after the perfusion, a 5.0 mL sample was withdrawn by syringe within a 5-min period, and the same volume of normal saline was simultaneously injected into the portal vein. Serum was prepared by centrifugation (10 min at 800 *g* at 4°C). A liver biopsy was performed immediately before and after the perfusion. To avoid degradation of β-C, the perfusion experiments were carried out in the dark. In all cases, compounds were infused into the jejunum in a single-pass mode. After perfusion, the animals were killed by puncturing the abdominal aorta. The perfused intestinal segments were removed, freed of their mesentery and serosal fat, and scraped with a glass slide. The intestinal mucosa scrapings were weighed and the length of the perfused intestine was recorded after drying. The liver was removed and weighed.

Extraction procedures

All samples were kept on ice and protected from the light. Extraction was performed immediately after the perfusion study for serum samples. Intestinal mucosa scrapings, lymph, and liver samples were stored under N_2 at −70°C and analyzed less than 1 month after the perfusion study.

Extraction of β-C isomers, retinol and retinyl esters. Two hundred mg of post-perfusion liver and 0.5 g of intestinal mucosa were homogenized in 5.0 mL of $CHCl_3$–CH_3OH 2:1 (vol/vol) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The Polytron probe was rinsed with 5 mL of $CHCl_3$–CH_3OH and the wash was added to the homogenate. Five mL of $CHCl_3$–CH_3OH 2:1 (vol/vol) was added to 0.5 mL serum and 0.8 mL lymph. Two mL of 0.9% sodium chloride was added to the samples. Echinenone and retinyl acetate in ethanol were added as internal standards in concentrations to approximate serum and tissue levels of carotenoids and retinoids, respectively. After centrifugation (10 min at 800 *g* at 4°C), the chloroform layer was saved and the residue was extracted again using 6.0 mL hexane. Chloroform and hexane layers were pooled and evaporated to dryness under N_2. The residue was redissolved in 100 μL ethanol. A 50-μL aliquot of the final extract was injected onto the HPLC system.

Extraction of RA isomers. A bond Elut™ aminopropyl column (500 mg/2.8 mL) and a Vac Elut™ vacuum elution apparatus were obtained from Analytichem International, Harbor City, CA. Two samples each of 0.5 g of intestinal mucosa scraping, 200 mg of pre-perfusion liver, and 200 mg of post-perfusion liver were homogenized separately as described above. Five mL $CHCl_3$–CH_3OH 2:1 (vol/vol) was added to two separate samples of 0.75 mL of serum. TMMP in methanol (100 μL, 10^{-7} M) was added as an internal standard. After the addition of 2 mL of 0.9% sodium chloride, the mixture was centrifuged (10 min at 800 *g* at 4°C). The chloroform layer was saved and the residue was extracted again using 5.0 mL of $CHCl_3$–CH_3OH 2:1 (vol/vol). The chloroform layers were pooled together, evaporated under N_2, and rediluted with 200 μL $CHCl_3$. This extract was applied to the aminopropyl column that was previously placed in the Vac Elut apparatus and washed with 2 mL hexane. The chloroform was pulled through the column by vacuum, and the constituents that were retained on the column were then eluted with 4 mL of chloroform–isopropanol 2:1 (vol/vol). This eluate consisted of neutral lipid and was discarded. The column was then eluted with 4 mL of diethyl ether with 3% acetic acid. The eluates from the same tissue (i.e., liver, intestine, or blood) were pooled together and were evaporated to dryness under N_2. The residue was redissolved in 100 μL methanol, and a 50-μL aliquot was injected onto the HPLC column.

HPLC analysis

β-carotene isomers, retinol and retinyl esters. The gradient reverse-phase HPLC system consisted of two Waters 510 pumps (Waters Chromatography Division of Millipore Corp., Mildford, MA); a Waters 490E multiwavelength spectrophotometer detector was set at 340 nm for retinoids and 455 nm for carotenoids, and an additional Waters 994 programmable photo diode array detector was used for measurement of maximal absorption. A Waters 715 ULTRA WISP autosampler was used for sample injection. Samples were analyzed by reverse-phase HPLC on a Vydac C18 reverse phase column (TP 201 Vydac Corp., Hesperia, CA) using CH_3CN–CH_3OH (solvent A, 45:55 (vol/vol) 0.1 mg/mL ammonium acetate in CH_3OH) and isopropanol (solvent B). The gradient procedure was as follows: 100% solvent A was used for 10 min followed by a 0.5 linear gradient to 40% solvent B, a 9.5-min hold at 40% solvent B, then a 2-min gradient back to 100% solvent A.

Retinoic acid isomers. The HPLC system consisted of a Series 410 LC pump, and LC-95 UV/visible spectrophotometer detector fixed at 340 nm at a maximum sensitivity (0.001 AUFS), an additional Waters 994 programmable photo diode array detector was used for measurement of maximal absorption. For sample injection, a Perkin-Elmer ISS-100 autosampler was used. Retinoic acid isomers were analyzed by reverse-phase HPLC on two Pecosphere™ 3 × 3CR ODS cartridge columns (Perkin-Elmer Corp., Norwalk, CT) using CH_3OH–H_2O (solvent A, 75:25 (vol/vol) 1% ammonium acetate in H_2O) and 100% CH_3OH (solvent B) as previously described (23). The lowest limit of detection for each assay was 0.2 pmol.

Calculations and statistics

Individual carotenoids and retinoids were identified by coelution with their standards and UV absorption spectra, and quantified relative to the recovery of internal standards, echinenone, retinyl acetate, and TMMP and by determining peak areas above observed baseline calibrated against known amounts of standards. Results are expressed as means ± SEM. Comparisons among the same group were performed using a one-tailed paired *t* test while comparisons among the different groups were performed using a one-tailed unpaired *t* test with the Bonferroni correction. β-carotene transport in lymph was calculated by regression analysis. Results were considered significantly different at $P < 0.05$.

RESULTS

The body weights of the animals (1.2 ± 0.1 kg, 1.3 ± 0.1 kg, and 1.1 ± 0.1 kg in Groups 1, 2, and 3,

TABLE 1. 9-*cis*-β-Carotene, all-*trans*-β-carotene, retinol, retinyl esters, 13-*cis*-, 9-*cis*-, and all-*trans*-retinoic acid concentrations in ferret intestinal mucosa

	Group 1 Micellar Solution	Group 2 9-*cis*-β-Carotene	Group 3 All-*trans*-β-Carotene
		pmol/g mucosa	
9-*cis*-β-Carotene	ND	1235 ± 409[a]	ND
All-*trans*-β-carotene	4 ± 2	6 ± 3	118 ± 31[a]
Retinol	100 ± 24[a]	265 ± 27	293 ± 45
Retinyl esters	2963 ± 1009	3463 ± 786	5117 ± 886
13-*cis*-Retinoic acid	0.9 ± 0.3	1.1 ± 0.3	1.1 ± 0.2
9-*cis*-Retinoic acid	1.1 ± 0.1	3.9 ± 0.5[a]	1.0 ± 0.3
All-*trans*-retinoic acid	5.8 ± 0.7	8.9 ± 1.0[a]	14.5 ± 0.9[a]

Concentrations were determined after 2 h perfusion of the micellar solution alone (Group 1, n = 3), 10 μM of 9-cis-β-carotene (Group 2, n = 4), or 10 μM of all-trans-β-carotene (Group 3, n = 4) for 2 h. Values (mean ± SEM) are expressed in pmol/g mucosa; ND, not detected.

[a]Significantly different ($P < 0.05$) from the other two groups.

respectively), weights of the intestinal mucosa after scraping (5.5 ± 0.5 g, 4.8 ± 0.5 g, 5.4 ± 0.2 g in Groups 1, 2, and 3, respectively), liver weights (29.7 ± 1.9 g, 30.5 ± 1.9 g, and 28.5 ± 1.3 g in Groups 1, 2, and 3, respectively), and the length of the perfused intestine (51 ± 2 cm, 52 ± 2 cm, and 53 ± 1 cm in Groups 1, 2, and 3, respectively) were similar among the different groups.

Intestinal uptake and lymphatic transport

The intestinal mucosa concentrations of 9-*cis*- and all-*trans*-β-C after perfusion are indicated in **Table 1.** The perfusion of the same amount of 9-*cis*- or all-*trans*-β-C resulted in a tenfold higher intestinal concentration of β-C after perfusion of the 9-*cis*- isomer ($P < 0.05$); 13-*cis*-β-C was not detected in the intestinal mucosa. After perfusion of either 9-*cis*- or all-*trans*-β-C, the intestinal concentrations of retinol and retinyl esters were not different regardless of the kind of isomer perfused. Although the concentrations of retinol and retinyl esters were higher than in the control group, the difference was significant ($P < 0.05$) only for retinol (Table 1).

The rates of lymph flow were similar in the three groups of ferrets and were 3.0 ± 0.3 mL/h, 3.5 ± 0.4 mL/h, and 3.4 ± 0.3 mL/h in Groups 1, 2, and 3, respectively. β-carotene was never detected in the lymph before perfusion and was not detected in the lymph during the 2-h perfusion of the micellar solution alone in the control group. In Groups 2 and 3, the perfusion of 10 μmol β-C resulted in a similar and linear increase in the amount of the perfused isomer (i.e., 9-*cis*- or all-*trans*-β-C) present in the 30-min lymph collection (**Fig. 1**). Using the slope from 0 to 120 min as an estimate of transport of β-C into

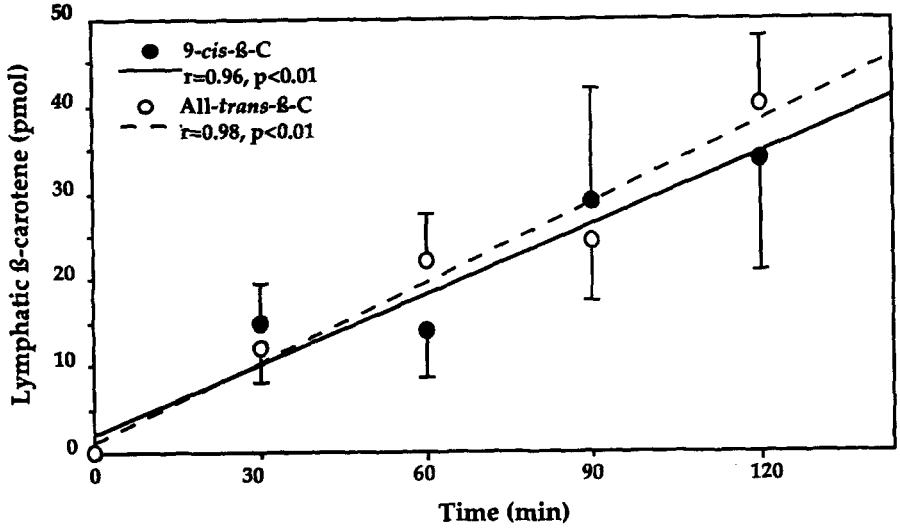

Fig. 1. Transport of intact β-carotene isomers into ferret mesenteric lymph (collected at 30-min intervals) during intestinal perfusion of 9-*cis*-β-carotene or all-*trans*-β-carotene at a concentration of 10 μM during 2 h. Results (mean ± SEM) represent the quantity (pmol) of β-carotene produced in each 30-min period. Values were fitted to a linear plot using linear regression analysis.

the lymph, the calculated rates were 18.3 ± 4.2 pmol/h during the perfusion of 9-*cis*-β-C and 19.8 ± 3.3 pmol/h during the perfusion of all-*trans*-β-C. The lymphatic transport of retinol and retinyl ester did not increase linearly after perfusion of β-C. Nevertheless, the lymphatic transport of retinol was significantly ($P < 0.05$) higher during the perfusion of 9-*cis*- or all-*trans*-β-C than in the control group (6.5 ± 0.8 nmol/h and 5.2 ± 0.7 nmol/h versus 1.9 ± 1.0 nmol/h in Groups 2, 3, and 1, respectively) (**Fig. 2**). The lymphatic transport of retinyl esters was also higher during the perfusion of β-C than in the control group but the difference was not significant. The lymphatic transport of either retinol or retinyl esters was not different regardless of the kind of isomer perfused (Fig. 2).

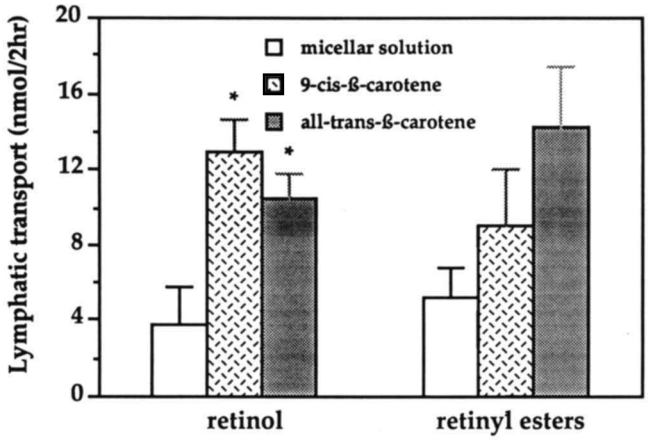

Fig. 2. Total accumulation of retinol and retinyl esters into ferret mesenteric lymph (collected at 30-min intervals) during the perfusion of the micellar solution alone (Group 1, n = 3), 10 μM of 9-*cis*-β-carotene (Group 2, n = 4), or 10 μM of all-*trans*-β-carotene (Group 3, n = 4) for 2 h. Results (mean ± SEM) are expressed in nmol/2 h.

Portal blood and liver concentrations of β-C, retinol, and retinyl esters

In all three groups of animals, similar and small amounts of all-*trans*-β-C were detected in the portal blood and their concentrations did not change after perfusion (**Table 2**). *Cis*-isomers of β-C were not detected in the portal blood before or after perfusion. In the three groups, retinol and retinyl ester concentrations in the portal blood were similar both before and after perfusion (Table 2). The concentrations of the different isomers of β-C detected in the liver of the ferrets after perfusion are presented in **Table 3.** No differences were observed among the groups and the *cis*- isomers of β-C represented 24.1 ± 2.1%, 25.6 ± 3.2%, and 20.8 ± 2.7% of the total amount of β-C in groups 1, 2, and 3, respectively. The

TABLE 2. All-*trans*-β-carotene, retinol, retinyl ester, 13-*cis*-, 9-*cis*-, and all-*trans*-retinoic acid concentrations in portal blood

	Group 1 Micellar Solution	Group 2 9-*cis*-β-Carotene	Group 3 All-*trans*-β-carotene
All-*trans*-β-carotene[a] (nmol/L)			
Before perfusion	12.4 ± 1.3	13.5 ± 1.8	12.8 ± 3.4
After perfusion	15.2 ± 2.2	13.3 ± 2.2	11.6 ± 3.4
Retinol (μmol/L)			
Before perfusion	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.2
After perfusion	1.0 ± 0.2	0.9 ± 0.3	0.9 ± 0.1
Retinyl esters (μmol/L)			
Before perfusion	6.6 ± 1.9	6.2 ± 1.8	9.4 ± 1.0
After perfusion	6.2 ± 1.6	6.2 ± 1.6	9.2 ± 2.4
13-*cis*-Retinoic acid (nmol/L)			
Before perfusion	1.0 ± 0.5	0.8 ± 0.3	0.9 ± 0.5
After perfusion	1.1 ± 0.5	0.8 ± 0.3	2.1 ± 0.8
9-*cis*-Retinoic acid (nmol/L)			
Before perfusion	0.7 ± 0.2	0.4 ± 0.1	0.5 ± 0.1
After perfusion	0.6 ± 0.1	2.6 ± 0.8[b]	0.7 ± 0.2
All-*trans*-Retinoic acid (nmol/L)			
Before perfusion	3.4 ± 0.5	3.5 ± 0.3	3.7 ± 0.4
After perfusion	3.6 ± 0.4	5.5 ± 0.4[c]	8.0 ± 1.6[c]

Concentrations were determined before and after perfusion of the micellar solution alone (Group 1, n = 3), 10 μM of 9-*cis*-β-carotene (Group 2, n = 4), or 10 μM of all-*trans*-β-carotene (Group 3, n = 4) for 2 h. Values are expressed as mean ± SEM.

[a] In all groups, *cis*- isomers of β-carotene were not detected in portal blood before or after perfusion.

[b] Significantly different from the two other groups at $P < 0.05$.

[c] Significantly different from Group 1 at $P < 0.05$.

TABLE 3. 13-*cis*-, 9-*cis*-, and all-*trans*-retinoic acid concentrations in liver before and after perfusion, and of all-*trans*-β-carotene, 9-*cis*-β-carotene, and 13-*cis*-β-carotene in ferret liver after perfusion

	Group 1 Micellar Solution	Group 2 9-*cis*-β-Carotene	Group 3 All-*trans*-β-Carotene
13-*cis*-Retinoic acid			
Before perfusion	2.2 ± 0.7	2.2 ± 0.4	2.5 ± 0.6
After perfusion	2.2 ± 0.8	2.1 ± 0.4	1.5 ± 0.3
9-*cis*-Retinoic acid			
Before perfusion	1.6 ± 0.3	6.0 ± 2.5	2.7 ± 0.7
After perfusion	1.3 ± 0.5	10.3 ± 2.6[a]	3.1 ± 0.7
All-*trans*-retinoic acid			
Before perfusion	17.5 ± 2.9	13.6 ± 3.3	10.7 ± 2.2
After perfusion	15.8 ± 3.7	19.6 ± 3.7	21.3 ± 3.7
All-*trans*-β-carotene			
After perfusion	68.3 ± 22.9	45.8 ± 10.2	42.5 ± 8.9
9-*cis*-β-Carotene			
After perfusion	12.3 ± 4.8	10.8 ± 1.8	7.8 ± 1.1
13-*cis*-β-Carotene			
After perfusion	10.6 ± 5.4	4.3 ± 1.9	2.5 ± 0.6

Concentrations were determined in ferret liver before and after perfusion of the micellar solution alone (Group 1, n = 3), 10 μM of 9-*cis*-β-carotene (Group 2, n = 4), or 10 μM of all-*trans*-β-carotene (Group 3, n = 4) for 2 h. Values are expressed as mean ± SEM in pmol/g wet liver.

[a] Significantly different from the two other groups at $P < 0.05$.

liver concentrations of retinol and retinyl esters after perfusion were not significantly different among the three groups of ferrets (data not shown).

Biosynthesis of retinoic acid

The intestinal mucosa concentrations of RA isomers after perfusion are indicated in Table 1, and the HPLC patterns detected after perfusing the micellar solution, 9-*cis* or all-*trans*-β-C are shown in **Fig. 3.** Small but similar concentrations of 13-*cis*-RA were detected in the three groups. After perfusion of 9-*cis*-β-C (Group 2), the intestinal mucosa concentration of 9-*cis*-RA was higher ($P < 0.01$) than in the two other groups, and all-*trans*-RA was significantly higher ($P < 0.05$) than in the control group. After perfusion of all-*trans*-β-C, only all-*trans*-RA was higher ($P < 0.01$) than in the control group. The all-*trans*-RA concentration in the intestinal mucosa was significantly higher ($P < 0.01$) after perfusion of all-*trans*-β-C than after perfusion of 9-*cis*-β-C (Table 1).

Before perfusion, the concentrations of the different isomers of RA in the portal blood were similar among the three groups (Table 2). The perfusion of the micellar solution in the control animals did not increase the portal blood concentration of the different isomers of RA (**Fig. 4A**). The perfusion of 9-*cis*-β-C resulted in a significant increase ($P < 0.05$) of both 9-*cis*- and all-*trans*-RA levels in portal blood (Fig. 4A). After perfusion of 9-*cis*-β-C the portal blood concentration of 9-*cis*-RA was significantly higher ($P < 0.05$) than in the other two groups, and the concentration of all-*trans*-RA was higher ($P < 0.05$) than in the control group (Table 2). The perfusion of all-*trans*-β-C increased ($P < 0.05$) only the portal blood concentration of the all-*trans*- isomer of RA (Fig. 4A). After perfusion of all-*trans*-β-C, the concentration of all-*trans*-RA was significantly higher ($P < 0.05$) than in the control group (Table 2). In the liver, the perfusion of 9-*cis*-β-C resulted in a significant increase ($P < 0.05$) of both 9-*cis*- and all-*trans*-RA levels (Fig. 4B) whereas the perfusion of all-*trans*-β-C increased ($P < 0.05$) only the liver concentration of the all-*trans*- isomer of RA (Fig. 4B). After 2 h perfusion, the 9-*cis*-RA concentration in the liver was significantly higher ($P < 0.05$) than in the other two groups (Table 3). The 9-*cis*-RA represented 7.9 ± 3.9% of the total liver concentration of RA in the control group, 13.7 ± 4.6% after perfusion of all-*trans*-β-C, and 32.1 ± 8.0% after perfusion of 9-*cis*-β-C ($P < 0.05$: group 2 vs. groups 1 and 3). In the portal blood and in the liver the net increase of all-*trans*-RA was higher after perfusion of all-*trans*-β-C than after perfusion of 9-*cis*-β-C, although the differences between the two groups did not reach the significance. After perfusion of all-*trans*-β-C, 13-*cis*-RA concentration increased slightly in portal blood (Fig. 4A) but not in the liver (Fig. 4B). However, these changes were not significant.

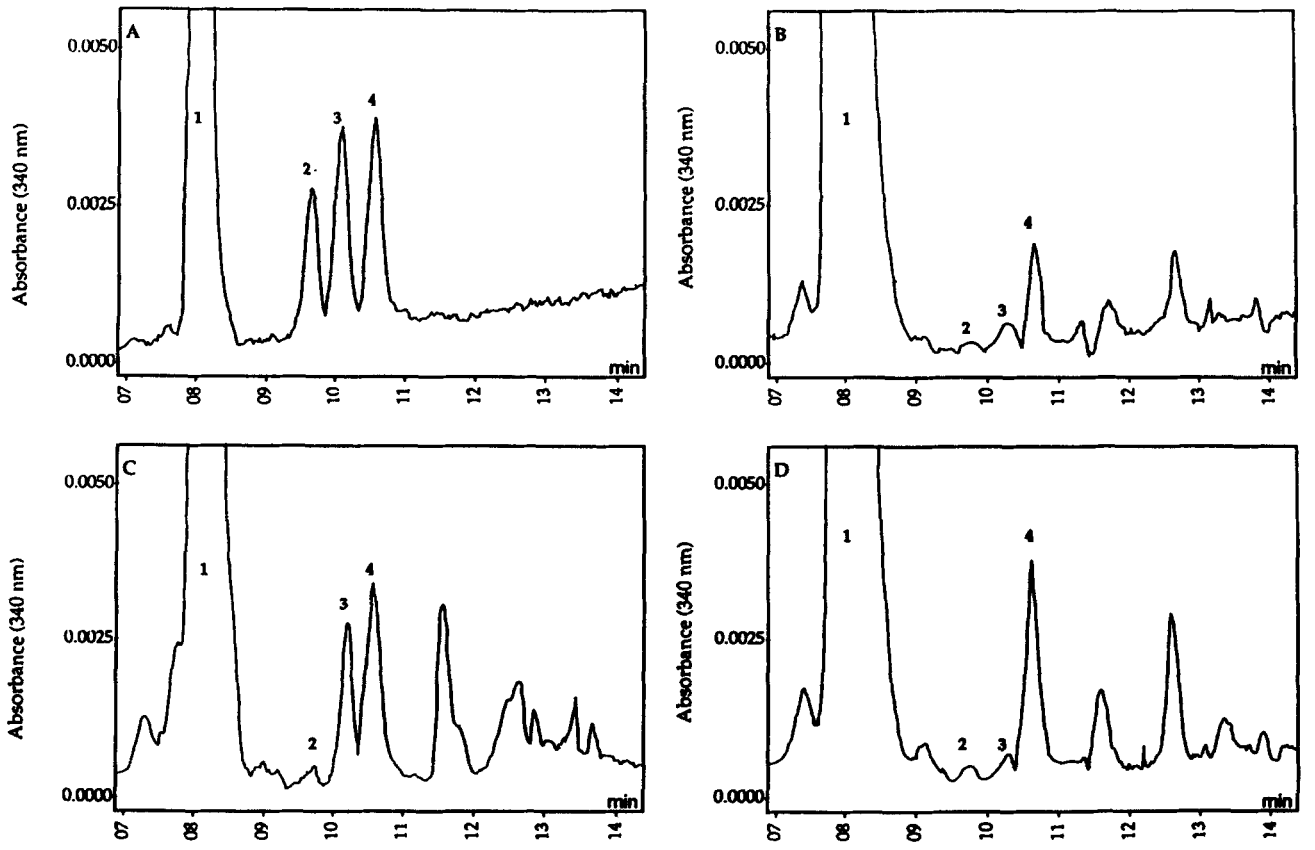

Fig. 3. HPLC chromatography of 13-*cis*-, 9-*cis*-, and all-*trans*-retinoic acid in ferret intestinal mucosa; (A) HPLC pattern after injection of standard retinoids; (B) after the intestinal perfusion of the micellar solution for 2 h in the control group; (C) after the intestinal perfusion of 10 μM of 9-*cis*-β-carotene for 2 h; (D) after the intestinal perfusion of 10 μM all-*trans*-β-carotene. Peak identifications: (1) TMMP added as internal standard (2) 13-*cis*-retinoic acid, (3) 9-*cis*-retinoic acid, (4) all-*trans*-retinoic acid.

DISCUSSION

Little is known about the bioavailability of 9-*cis*-β-C. However, the lack of serum response after supplementation by 9-*cis*-β-C in humans (16–18) suggests that this isomer is poorly absorbed. Additionally, Jensen et al. (16) reported that in human serum the *trans*-/*cis*- ratio of β-C is about ten, suggesting that the intact *trans*- form of β-C is preferentially absorbed. In contrast, Rushin, Catignani, and Schwartz (14) showed a *trans*-/*cis*- ratio of β-C of about three in fresh serum samples, which is close to the ratio found in fruits and vegetables (9). Stahl et al. (15) demonstrated the presence of substantial amounts of 9-*cis*- and all-*trans*-β-C in human tissue samples obtained by autopsy. In this study, the liver concentration of 9-*cis*-β-C exceeded 25% of the total amount of the β-C, while no 9-*cis*-β-C and only small amounts of 13-*cis*- were detected in the serum. These observations raise the question of the source of 9-*cis*-β-C in the body. It is well known that *cis*-carotenes are present in food (13) and processed vegetables contain as much as 50% of their total β-C as *cis*-isomers (9). Moreover, biostereoisomerization of all-*trans*-carotenes probably occurs naturally in the gastric and/or intestinal lumen due to several factors such as heat, gastric acid pH, and/or intestinal microflora (11). After the administration of different carotenoids to chicks, Deuel et al. (12) found that 50–70% of the recovered pigment showed a *cis*-configuration in the feces, gut washing, and liver. If the *cis*- form of the molecule can be absorbed, food may be an important source of 9-*cis*-β-C for the body; if not, the presence of this isomer in several tissues could be explained by the isomerization of the all-*trans*- form after intestinal absorption.

In the present experiments we demonstrated that both all-*trans*-β-C and 9-*cis*-β-C are absorbed through the lymphatic system after intestinal perfusion in the ferret. After perfusion of either 9-*cis*- and all-*trans*-β-C, the amount of the corresponding β-C isomer increased linearly in the lymph (Fig. 1) which supports that β-C absorption takes place by passive diffusion (31), as the study was done under initial rate conditions. The similarity of the two straight lines obtained with different isomers of β-C (Fig. 1) is striking and demonstrates that there is no preferential transport through the lymph regardless of the kind of isomer perfused. Retinol and retinyl esters did not in-

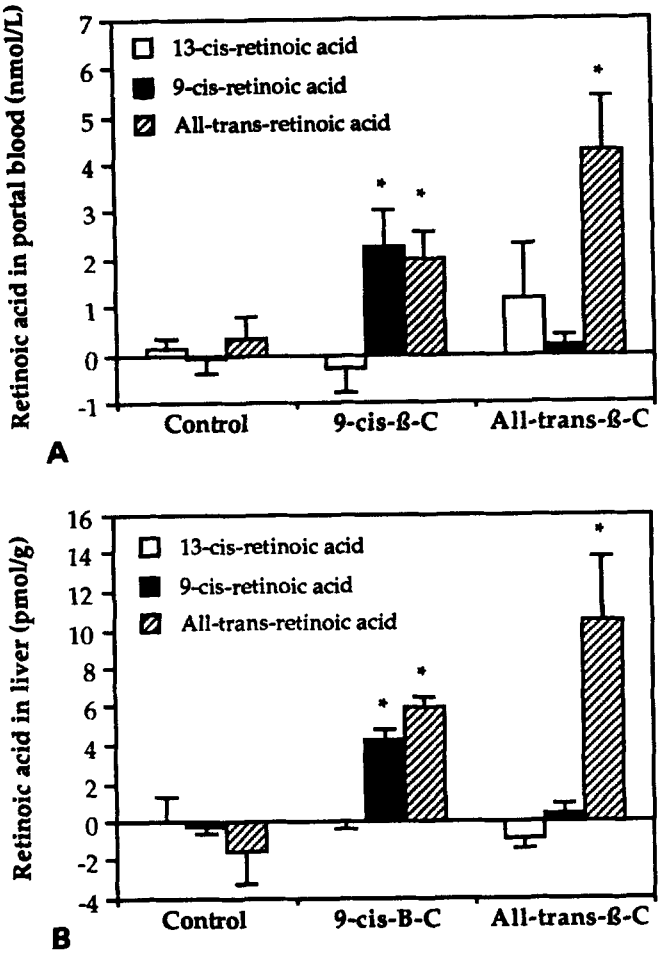

Fig. 4. The net increase of retinoic acid from baseline (mean ± SEM) in portal vein blood (A) and in liver (B) of ferrets during the perfusion of the micellar solution alone (Group 1, n = 3), 10 μM of 9-*cis*-β-carotene (Group 2, n = 4), or 10 μM of all-*trans*-β-carotene (Group 3, n = 4) for 2 h. *Significantly different from baseline value at $P < 0.05$.

crease linearly in the 30-min lymph collections. This may be the due to the absorption of varying amounts of residual retinol and retinyl ester as we did not use vitamin A-depleted animals, and to the high retinyl ester status in the ferret (32). Nevertheless, the lymph transport of retinol was higher in the β-C-perfused animals than in the control group, but was similar between the two isomer types perfused.

An important aspect of this study is the demonstration that after perfusion of β-C, the intestinal uptake is about tenfold higher after perfusion of the 9-*cis*- isomer compared to the pure all-*trans*- isomer. It seems unlikely that 9-*cis*-β-C simply adhered to the intestine as intestinal mucosa was carefully washed before homogenization and the same procedure was used for the perfusion of the two isomers. Moreover, in the case of adherence, a much higher recovery of β-C would be expected. After 9-*cis*-β-C perfusion, the total β-C uptake by the perfused intestinal mucosa was only about 5 nmol compared to the 2 μmol of β-C perfused. The increased uptake of the 9-*cis*-β-C compared to the all-*trans*- is consistent with the results of Ben-Amotz et al. (19). Feeding chicks and rats with synthetic all-*trans*-β-C or with β-C derived from the algae, *Dunaliella bardawil*, which is composed of about equal amounts of the all-*trans*- and 9-*cis*- isomers, they reported a tenfold higher accumulation of the algae β-C isomer mixture in the liver than of the synthetic all-*trans*-β-C. Because of its planar structure, an all-*trans*-carotenoid is less soluble, more stable and more likely to crystallize than its *cis*- isomers (11). The preferential intestinal uptake of 9-*cis*-β-C may be the result of these different chemical properties, but could solely be the result of the non-planar structure of the *cis*- isomer, which might favor the pick up by the intestinal brush border from the micelles. The incorporation of β-C into chylomicrons and their exocytosis into the lymph seems not to be affected by the stereochemistry of the β-C, as the lymphatic transport of the two isomers was similar. The demonstration of the absorption of 9-*cis*-β-C into the lymph raises the question why non-responses have been seen in human serum after supplementation with this isomer (16–18). A species-related difference is unlikely because like the human (33), the ferret is able to absorb intact β-C into lymph (28), and, as reported in humans (15), we observed that 25% of the β-C concentration in the ferret liver is the 9-*cis*-isomer, although blood samples are devoid of *cis*-β-C. We suggest that, after its absorption, 9-*cis*-β-C has a more rapid peripheral tissue uptake than all-*trans*-β-C. The high intestinal affinity of 9-*cis*-β-C may be an argument for this hypothesis. Interestingly, in our previous study (28) in ferrets, after perfusion of natural β-C (about 25% 9-*cis*- and 75% all-*trans*-β-C) under similar experimental conditions the intestinal concentration of β-C was about 1 nmol/g, with the same *cis*-/*trans*- ratio as in the original micellar solution (unpublished data) and, again, tenfold higher than the result obtained with the >98% pure, synthetic all-*trans*-β-C. In rat livers, Ben-Amotz et al. (19) showed that the accumulation of all-*trans*-β-C was higher when 9-*cis*-β-C was also provided in the diet. These observations suggest that the intestinal uptake of all-*trans*-β-C may be stimulated by the presence of the 9-*cis*- isomer and it was argued that the 9-*cis*- isomer may serve as a good solvent for the *trans*- isomer (19).

The methodology of this study does not allow us to determine whether the luminal phase of the absorption of β-C is influenced by the *cis*- or *trans*- forms of the molecule because, in both groups, the micellar solution was artificially made in the laboratory by sonication. As previously described (28, 29), no intact β-C was absorbed through the portal blood. Because the lymph was collected, it was expected that the liver concentration of β-C after perfusion would be similar among the three groups.

Similarly, it is not surprising to see that portal and liver concentrations of retinol and retinyl esters did not change. However, in the two β-C groups, after perfusion, the intestinal concentrations of retinol and retinyl esters were similar and higher than in the control group (Table 1) demonstrating a comparable synthesis of vitamin A by these two β-C isomers. Unfortunately, with the HPLC system used, we were not able to resolve *trans*-retinol and *trans*-retinyl ester from their *cis*- isomers.

In the first part of this study we demonstrated the intestinal uptake and the lymphatic transport of 9-*cis*-β-C after intestinal perfusion in the ferret. Since the discovery of 9-*cis*-RA as a specific ligand for the RXR nuclear receptor (25–27) the question arose about the source of 9-*cis*-RA in the body, which may be produced by isomerization of all-*trans*-RA or by cleavage of 9-*cis*-β-C. We have recently demonstrated that, after in vitro incubations with intestinal homogenates, the intestinal cleavage of 9-*cis*-β-C can be a source of 9-*cis*-RA (34). However, this effect may not take place in vivo, or the 9-*cis*-RA formed by intestinal cleavage may not be available for absorption. In 1967, Crain, Lotopeich, and Krause (35) first described the conversion of β-C into RA by the rat intestinal mucosa and the transport of this polar compound via the portal vein. It is well known that β-C is a precursor of RA and we have shown that the intestinal perfusion of β-C in the ferret raises RA levels in portal blood (29).

In the second part of the study we demonstrated in vivo that the intestinal cleavage of 9-*cis*-β-C can provide a source of 9-*cis*-RA for the body. Only small amounts of 9-*cis*-RA were detected in the intestinal mucosa in the control group and after perfusion of all-*trans*-β-C. However, substantial amounts of 9-*cis*-RA were detected after perfusion of 9-*cis*-β-C (Table 1), thus demonstrating the in vivo biosynthesis of 9-*cis*-RA by intestinal cleavage of 9-*cis*-β-C. As in the control group, the intestinal perfusion of all-*trans*-β-C did not increase the 9-*cis*-RA concentrations in portal blood or liver. In contrast, the intestinal perfusion of the same concentration of 9-*cis*-β-C resulted in a significant increase of the 9-*cis*-RA concentration in the portal blood (Fig. 4A) and in the liver (Fig. 4B) demonstrating the absorption via the portal vein of the 9-*cis*-RA that was formed by intestinal cleavage of 9-*cis*-β-C. Interestingly, the amounts of 9-*cis*- and all-*trans*-RA formed by cleavage of 9-*cis*-β-C were very close in the portal blood (Fig. 4A) and in the liver (Fig. 4B). In the intestine, if we use the amount of RA obtained in the control group as a baseline for the other groups, the amount of 9-*cis*- versus all-*trans*-RA formed was about 2.8 versus 3.1 pmol/g. Moreover, the total amounts of RA formed after perfusion of 9-*cis*- versus all-*trans*-β-C were still very close (respectively, 4.1 vs. 4.3 nmol/L in the portal blood, 10.2 vs. 10.5 pmol/g in the liver, and approximately 5.9 vs. 8.7 pmol/g in the intestine). These results are consistent with our previous in vitro study, wherein the biosynthesis of 9-*cis*- and all-*trans*-RA from 9-*cis*-β-C was similar and linear with increasing concentrations of β-C (34). The present study does not allow us to determine whether the cleavage of the 9-*cis*-β-C was primarily central or excentric (36, 37). Although we previously demonstrated that 9-*cis*-retinal may be a source of 9-*cis*-RA (34), the formation of 9-*cis*-RA may occur from a series of 9-*cis*-β-apocarotenals.

The possibility of *cis-trans* isomerization in vivo may have functional significance. The in vivo isomerization of 13-*cis*-RA to all-*trans*-RA has been demonstrated by McCormick, D'Ortona Kroll, and Napoli (38) while evidence of in vivo formation of 13-*cis*-RA from all-*trans*-RA was obtained by Zile, Inhorn, and DeLuca (39). In our experimental in vivo study we did not find evidence for the bioisomerization of all-*trans*-RA to 9-*cis*- or 13-*cis*-RA. Although after perfusion of all-*trans*-β-C, the concentration of 13-*cis*-RA increased slightly in the portal blood (Fig. 4A) this change was not significant, and 13-*cis*-RA concentration did not change in the liver or in the intestine. However, if only a small percentage of all-*trans*-RA is isomerized to 13-*cis*-RA, the observation of the formation of 13-*cis*-RA after perfusion of all-*trans*-β-C in vivo would require an extremely sensitive system of detection and this process cannot be ruled out by our experiments.

In summary, this study has demonstrated the intestinal uptake and the lymphatic transport of 9-*cis*-β-C as well as the biosynthesis of 9-*cis*-RA from 9-*cis*-RA in vivo after intestinal perfusion in the ferret. Therefore, we suggest that alimentary 9-*cis*-β-C may be a substantial source of 9-*cis*-β-C and 9-*cis*-RA for the body, playing an important role in regulating cell function.

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