All-*trans* β-Carotene Is Absorbed Preferentially to 9-*cis* β-carotene, but the Latter Accumulates in the Tissues of Domestic Ferrets (*Mustela putorius puro*)[1,2,3]

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ABSTRACT The algae *Dunaliella bardawil* and *Dunaliella salina* naturally contain large concentrations of all-*trans* and 9-*cis* β-carotene (βC). The purpose of this study was to compare the relative serum and tissue accumulation of all-*trans* and 9-*cis* βC in ferrets fed different ratios of all-*trans*/9-*cis* βC derived from two commercial sources, *D. bardawil* or *D. salina* (Betatene). Male ferrets (7 wk old) were fed carotene-free, pelleted diets for 27 d. Beginning on d 18, groups of ferrets (n = 6 or 7) received daily, one of six oral supplements varying in ratios of 9-*cis* and all-*trans* βC mixed with ~1.0mL of Ensure. Four supplements containing 5.2–8.3 μmol total βC were prepared from a 20% Betatene preparation, *D. bardawil*, a high-*cis* Betatene preparation, and Betatene further enriched in 9-*cis* βC with all-*trans* βC/9-*cis* βC ratios of 2.2, 1.5, 0.6 and 0.4, respectively. Two control supplements, high and low βC, were prepared from commercial βC beadlets. The high control supplement had an all-*trans*/9-*cis* ratio of 19.0, whereas 9-*cis* βC was not detected in the low supplement. On d 27, serum and tissues were obtained for HPLC analysis of βC and its isomers. Analysis of livers showed that all-*trans* βC was the primary isomer present, but 9-*cis* and other isomers were also detected in all groups. The hepatic all-*trans*/9-*cis* ratios were 5.9, 4.9, 2.5, 1.4, 52.2 and 47.5, respectively, for the groups listed above. Lower amounts of all-*trans* and 9-*cis* βC were found in kidneys compared with the liver, but ratios of all-*trans*/9-*cis* were not different among groups. Only trace amounts of 9-*cis* βC were found in serum. These results demonstrate that the algae *D. bardawil* and *D. salina* provide a bioavailable source of βC isomers, but, as in humans, absorption of 9-*cis* βC is poor and any 9-*cis* βC absorbed is apparently cleared by the liver. J. Nutr. 128: 2009–2013, 1998.

KEY WORDS: • 9-*cis* β-carotene • domestic ferrets • carotenoids • all-*trans* β-carotene

There are ~600 naturally occurring carotenoids, although it is not clear how many have biological value in humans. β-Carotene (βC),[5] the carotenoid that gives carrots their orange color, is the most commonly studied carotenoid due to its abundance in foods, its antioxidant properties and its nutritional value as a precursor of vitamin A (VA). βC exists primarily with all of its double bonds in the *trans* configuration. However, light and heat can cause isomerization of one or more double bonds to *cis* configurations. There are numerous possible isomers of each carotenoid; however, the number of stable geometric isomer forms is limited. The primary isomers of βC found in foods are all-*trans*, 9-*cis* and 13-*cis* (Chandler and Schwartz 1987).

Retinoid binding to nuclear receptors is important for growth, reproduction and maintenance of the skin and mucous membranes. In in vitro studies, 9-*cis* retinoic acid was determined to be a ligand for the human nuclear retinoic acid receptor, RXR-α (Heyman et al. 1992, Levin et al. 1992). 9-*cis* Retinoic acid can be formed by the oxidation and isomerization of dietary retinol. In addition, 9-*cis* βC can also be a source of 9-*cis* retinoic acid (Nagao and Olson 1994, Wang et al. 1994).

The potential conversion of 9-*cis* βC to the ligand 9-*cis* retinoic acid has led to increased interest in the absorption and metabolism of 9-*cis* βC. Stahl and co-workers (1993) found that when subjects ingested an isomer mixture of carotenoids, serum levels of α-carotene, all-*trans* βC, and other isomers of βC such as 13- and 15-*cis* increased, but 9-*cis* βC did not. Other researchers have also noted discrimination in absorption and transport of 9-*cis* vs. all-*trans* βC (Gaziano et al. 1995, Stahl and Sies 1994). Even though humans have very low serum 9-*cis* βC concentrations when fed a dose of 9-*cis* βC,

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[5] Abbreviations used: (βC), β-carotene; RXR, retinoid X receptor; VA,vitamin A.

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relatively high levels are found in the tissues (Stahl et al. 1992 and 1993). It is unclear whether this is due to rapid clearing of absorbed 9-*cis* βC into tissues, tissue isomerization of all-*trans* βC, or some other unknown mechanism. The study of 9-*cis* βC metabolism in humans is difficult, although a human study with ^{13}C-9 *cis* βC suggested substantial isomerization of 9-*cis* βC to all-*trans* βC in the gastrointestinal tract before absorption (You et al. 1996). Animal models that absorb βC similarly to humans should provide tissue data not achievable in human studies.

Many species, such as rats, do not accumulate βC in tissues when βC is fed at physiologic levels (Erdman et al. 1993). Like humans, domestic ferrets (*Mustela purorius furo*) have been shown to absorb substantial portions of ingested βC intact and to accumulate βC in tissues (Gugger et al. 1992, Lederman et al. 1998, Ribaya-Mercado et al. 1989). Previous studies in this laboratory have found that the ferret is an appropriate animal model to study the absorption and tissue uptake of βC and its isomers (Lederman et al. 1998, White et al. 1993, Zhou et al. 1996). The purpose of this study was to compare the relative serum and tissue accumulation of all-*trans* and 9-*cis* βC in ferrets fed different ratios of all-*trans*/9-*cis* βC derived from two commercial sources, *Dunaliella bardawil* or *Dunaliella salina* (Betatene).

METHODS AND MATERIALS

Animals and diets. Male, descented 8-wk-old ferrets (n = 40) were purchased from Marshall Farms (North Rose, NY). Upon arrival, all ferrets were individually housed in stainless steel rabbit cages. Ferrets had free access to food and water. Food intake, body weight and health were monitored.

Ferrets were fed a semipurified, pelleted diet (Research Diets, New Brunswick, NJ), which has been used successfully in previous ferret studies (Lederman et al. 1998, White et al. 1993, Zhou et al. 1996). The diet contained 5.4 mg/kg diet vitamin A (as retinyl palmitate) and undetectable levels of βC or other carotenoids (+VA/-βC diet). Oral doses of various all-*trans* and 9-*cis* βC supplements were prepared from two different sources, *D. bardawil* and *D. salina* (Betatene). All diets were stored at 4°C until use.

Experimental design. Upon arrival, ferrets (n = 40) were fed the basal (+VA/-βC) diet for 17 d to reduce body stores of βC (White et al. 1993) and to allow acclimation to the new environment. Vitamin A was included in the diet to maintain adequate VA status. After acclimation, ferrets were separated into six experimental groups (n = 6 or 7) and continued to receive the basal diet. Beginning on d 18, each group received a daily oral βC supplement containing βC mixed with lecithin in ~1.0 mL Ensure (Ross Laboratories, Columbus, OH). The doses, containing from 5.2 to 8.3 μmol total βC, were prepared from the following: Betatene (Betatene A), high-*cis* Betatene (Betatene B) (both gifts from Henkel, LaGrange, IL), high-*cis* Betatene further enriched in 9-*cis* βC (Betatene C), *D. bardawil*, or βC beadlets (a gift from Hoffmann LaRoche, Nutley, NJ) (high βC control) with ratios of all-*trans* βC/9 to *cis* βC of 2.2, 0.6, 0.4, 1.5 and 19.0, respectively. The final group received a low βC control dose, prepared from βC beadlets, which contained 0.8 μmol all-*trans* βC and no detectable 9-*cis* βC (**Table 1**).

After 9 d of dosing, the ferrets were anesthetized with a mixture of ketamine hydrochloride (Vetalar, Parke-Davis, Morris Plains, NJ) 11–15 mg/kg body weight and xylazine (Rompun, Miles Laboratories, Shawnee, KS) 0.3–1.0 mg/kg body weight administered by intramuscular injection. Blood samples were collected by cardiac puncture, and the ferrets were killed by severing the brachial vessels between the pectoralis major and the latissimus dorsi. The liver, kidneys and adrenal glands were then dissected and weighed. Tissue and serum samples were stored at −20°C until analysis, which was completed within 6 mo. All procedures were approved by the University of Illinois Laboratory Animal Care Advisory Committee.

All procedures were carried out under yellow lighting.

TABLE 1

Sources and β-carotene (βC) composition of daily oral supplements fed to ferrets for 9 d[1]

Source	all-*trans* βC	9-*cis* βC	Ratio, a-t:9-c[2]
		μmol	
Betatene A[3]	5.7	2.6[3]	2.2
Betatene B	2.0	3.2	0.6
Betatene C	1.9	5.4	0.4
Dunaliella bardwil	3.7	2.4	1.5
βC beadlets (high control)	5.7	0.3	19.0
βC beadlets (low control)	0.8	ND[4]	—

[1] Supplemental doses consisted of the supplement mixed with Ensure and lecithin, and were administered once daily for 9 d.
[2] a-t, all-*trans* βC; 9-c, 9-*cis* βC.
[3] See text for description of sources.
[4] 9-*cis* βC was not detected.

Dose preparation and administration. The βC doses were prepared daily and isomeric content verified by HPLC. Mean values of this analysis are presented in Table 1. The samples were mixed with Ensure because previous studies in this laboratory have shown that ferrets will readily drink this mixture (White et al. 1993). Lecithin (~1.0 mL) was added to 12 mL of Ensure before addition of the various preparations to allow emulsification of the βC in the Ensure mixture. Water bottles were removed each morning and returned immediately after the dose was administered (early afternoon). The 1.0-mL dose was administered in a disposable pipet placed directly into the mouth. Doses were consumed quickly, without difficulty.

Betatene A, Betatene B (both gifts from Henkel), and *D. bardawill* (spray dried algae powder, a gift from NBT Eliat, Israel) were used as obtained from the supplier. To prepare the Betatene C dose, ~0.3g of Betatene B was vortexed with 10 mL ethanol (with 1.0 g/L BHT) for 1min. The mixture was centrifuged in a benchtop centrifuge for 5 min to pellet the unsolubilized portion and the ethanol was removed and placed into a glass tube. One milliliter of KOH (600 g/L water) was added to the ethanol and the mixture was saponified for 20 min at 70°C. Deionized water (1 mL) and 200 μL of 1mol/L KCl were added and the mixture was placed on ice to cool. Once cooled, 15.0 mL of hexane (with 1.0 g/L BHT) was added, the mixture was vortexed for 1 min and the hexane layer was removed. The hexane extraction was repeated three times. The hexane extracts were combined and evaporated under vacuum.

Serum and tissue preparation. Tissues were saponified and βC was extracted from tissues and serum as previously described by our laboratory (Lederman et al. 1998). Saponification does not alter the all-*trans*/9-*cis* βC ratio in tissues (data not shown).

Preparation of intestinal mucosal samples. The small intestine of one ferret supplemented with Betatene C was removed and thoroughly rinsed with ice-cold saline (9.0 g/L NaCl) before being sliced longitudinally. The mucosal layer was then scraped with a glass slide in 20-cm increments from duodenum to ileum. The weight of each increment was recorded. Mucosal scrapings were extracted and analyzed by HPLC for βC as described for tissue samples.

HPLC analysis. Quantification of βC by HPLC was performed as described by Lederman et al.(1998).

Statistical analysis. All analyses were compared between groups using one-way ANOVA and Fishers Protected Least Significant Difference analysis (StatView 512$^+$, Brain Power, Calabasas, CA). Differences were considered significant at $P < 0.05$. Data shown represent group means ± SD. Linear regression was performed using CA-Cricket Graph III version 1.0 (Computer Associates International, Islandia, NY).

RESULTS

There was no effect of carotenoid dosing on food intake, body weight or apparent health of the ferrets (data not

TABLE 2

Hepatic concentrations of all trans (a-t) and 9- cis (9-c) β-carotene (βC), and percentage recovery of all-trans and 9-cis βC from liver after 9 d of oral supplementation of ferrets with algal extracts of all-trans and 9-cis β-carotene[1,2]

Group	Liver βC		a-t/9-c	doses recovered	
	all-*trans*	9-*cis*	ratio	all-*trans*	9-*cis*
	nmol/g			%	
Betatene A	5.3 ± 1.4[a]	0.9 ± 0.1[b]	5.9	3.5	1.2
Betatene B	2.0 ± 0.4[c]	0.8 ± 0.2[b]	2.5	3.7	0.8
Betatene C	1.5 ± 0.5[c]	1.1 ± 0.3[a]	1.4	2.6	0.7
Dunaliella bardwil	3.9 ± 0.7[b]	0.8 ± 0.2[b]	4.9	3.7	1.2
βC beadlets (high control)	4.7 ± 1.7[a,b]	0.09 ± 0.04[c]	52.2	3.0	1.0
βC beadlets (low control)	1.9 ± 0.5[c]	0.04 ± 0.02[c]	47.5	8.2	ND[3]

[1] Values represent group means ± SD, $n = 6$ or 7. Values in a column with no superscripts in common are significantly different ($P < 0.05$).
[2] See text for supplement composition.
[3] 9-*cis* βC was not detected.

shown). Because the liver is the major site of accumulation for dietary βC and vitamin A in ferrets (Ribaya-Mercado et al. 1989, White et al. 1993), liver stores of all-*trans* and 9-*cis* βC were used as the primary indicator of βC absorption and storage. Under similar experimental conditions, we found low conversion efficiency of βC to VA (< 15:1) (Lederman et al. 1998). Thus, accumulated hepatic βC should account for most of the βC absorbed from diet.

The predominant βC isomer in the livers was all-*trans* for all groups (**Table 2**). Liver concentrations of all-*trans* βC were significantly higher in the Betatene A group than in all other groups except the βC beadlet high control group. The concentration of 9-*cis* βC in the liver was significantly higher in the Betatene C group compared with any other group, whereas both the βC beadlet control groups had significantly lower hepatic 9-*cis* βC than all other groups. For all groups except the low βC control, the liver had a higher all-*trans*/9-*cis* βC ratio than that of the supplement provided (Tables 1 and 2). Liver stores of all-*trans* and 9-*cis* βC were both positively correlated with the amount in the daily supplemental dose; however, the liver stores of all-*trans* βC increased at a substantially higher rate than 9-*cis* as the amount in the oral dose increased (**Fig. 1**). The percentage of βC provided in the 9 d of supplemental doses recovered in the liver was between 2.6 and 8.2% for all-*trans* βC, but only 0.7–1.2% for 9-*cis* βC (Table 2).

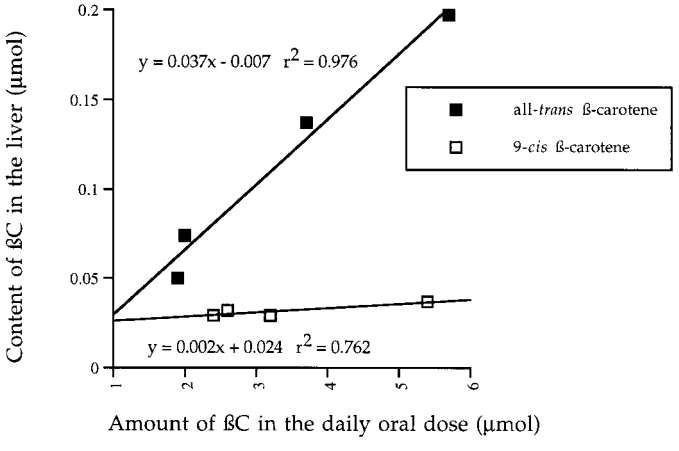

FIGURE 1 The amount of all-*trans* and 9-*cis* β-carotene (βC) in the liver of ferrets is positively correlated with the amount consumed in a daily supplement.

Serum from ferrets fed Betatene A, *D. bardawil* or high βC control had higher concentrations of βC than the other groups (**Table 3**). As with the liver, there was a linear relationship between the amount of all-*trans* βC in the diet and the amount in the serum ($r^2 = 0.77$) (data not shown). Serum levels of 9-*cis* were below detection levels or very low. Only four ferrets from the Betatene C and two ferrets from the *D. bardawil* groups had detectable levels of 9-*cis* βC (data not shown). The lower limit of detection of 9-*cis* βC was 0.004 nmol/L serum.

The ferret kidneys and adrenals had a pattern of all-*trans* and 9-*cis* βC concentrations similar to that seen in the livers (Table 3). However, in most groups, the ratio of all-*trans*/9-*cis* βC in these tissues was substantially higher than the ratios in the diet or in the liver.

The small intestine of a single ferret, dosed with Betatene C with a ratio of all-*trans* to 9-*cis* βC of 0.4, was removed at the termination of the study. Nine sequential segments (section one beginning at the duodenum and section nine ending at the ileum) were analyzed for the concentration of all-*trans* and 9-*cis*, βC (**Fig. 2**). There was substantially more all-*trans* than 9-*cis* βC in the mucosal cells obtained from every segment of the intestine. Mucosal cells from the first four segments contained higher amounts of βC than the latter five. The 9-*cis* βC isomer was not detectable in the last two segments of the intestine.

DISCUSSION

Several studies have analyzed human serum for isomers of β-carotene. Stahl and co-workers (1993) reported that the predominant βC isomer in human serum is all-*trans* and that only negligible amounts of 9-*cis* βC are found, suggesting that there may be preferential uptake and absorption of all-*trans* βC compared with the 9-*cis* βC isomer. In contrast, this same group analyzed the concentrations of βC and lycopene isomers in selected human tissues and found considerable levels of 9-*cis* βC along with the other βC isomers (Stahl et al. 1992 and 1993). The *cis* isomers of βC are reported to comprise 15–30% of the βC in tissues, but constituted only ~5% of the βC in serum (Stahl and Sies 1994).

Gaziano et al. (1995) found that humans supplemented

TABLE 3

Concentration of all-trans (a-t) β-carotene (βC) and 9-cis βC in the serum and tissues of ferrets after 9 d of oral supplementation with algal extracts of all-trans and 9-cis β-carotene[1]

Group	Kidney		a-t/9-c ratio	Adrenal		a-t/9-c ratio	Serum[2]
	all-*trans*	9-*cis*		all-*trans*	9-*cis*		all-*trans*
	nmol/g			*nmol/g*			*nmol/L*
Betatene A[3]	0.74 ± 0.34[a]	0.04 ± 0.01[a,b]	18.5	1.54 ± 0.91[a]	0.06 ± 0.05[a]	25.7	0.30 ± 0.11[a]
Betatene B	0.33 ± 0.09[c]	0.03 ± 0.01[b]	11.0	0.63 ± 0.23[b,c]	0.02 ± 0.04[a,b]	31.5	0.12 ± 0.04[b]
Betatene C	0.27 ± 0.11[c]	0.04 ± 0.01[a,b]	6.8	0.54 ± 0.22[c]	0.03 ± 0.02[a,b]	18.0	0.11 ± 0.05[b]
Dunaliella bardawil	0.55 ± 0.11[b]	0.05 ± 0.03[a]	11.0	1.62 ± 0.76[a]	0.05 ± 0.04[a,b]	32.4	0.25 ± 0.09[a]
βC beadlet (high control)	0.64 ± 0.14[a,b]	0.04 ± 0.01[a,b]	16.0	1.24 ± 0.54[a,b]	0.02 ± 0.04[a,b]	62.0	0.22 ± 0.10[a]
βC beadlet (low control)	0.28 ± 0.09[c]	0.03 ± 0.01[b]	9.3	0.66 ± 0.26[b,c]	0.01 ± 0.04[b]	66.0	0.12 ± 0.05[b]

[1] Values represent group means ± SD, $n = 6$ or 7. Values in a column with no superscripts in common are significantly different ($P < 0.05$).
[2] 9-*cis* βC was not detected in the serum.
[3] See text for supplement composition.

with a 50/50 mixture of 9-*cis* and all-*trans* βC had serum increases in both isomers; however, the change in serum 9-*cis* βC accounted for only a small portion of the total βC change. They observed the same changes in subjects supplemented with only all-*trans* βC, suggesting an absorption discrimination between βC isomers. The presence of 9-*cis* βC in the tissues of animals fed a diet containing little 9-*cis* βC may also be due to isomerization of the all-*trans* isomer to 9-*cis* before or during absorption, or within tissues.

Very low levels of 9-*cis* βC are seen in the serum of individuals fed pure 9-*cis* βC, possibly due to isomerization of 9-*cis* βC into all-*trans* βC. This isomerization may occur in the gastrointestinal tract, in the mucosal cells or after absorption. Tamai and co-workers (1995) fed either a mixture of 9-*cis* βC or pure all-*trans* βC to subjects for 44 wk and found that serum levels of 9-*cis* βC were highest in the group fed only the all-*trans* βC. Stahl et al. (1993) suggested that a tissue isomerase that converts 9-*cis* βC into the all-*trans* form may be present. The most supportive evidence of isomerization of 9-*cis* βC to all-*trans* βC is provided by You and co-workers (1996). In that study, humans were fed a dose of highly purified (99.9%) ^{13}C-labeled 9-*cis* βC. Analysis of the ^{13}C-βC in the blood revealed substantial quantities of ^{13}C all-*trans* βC but only negligible amounts of ^{13}C 9-*cis* βC. It is possible that some 9-*cis* βC is converted to all-*trans* βC in the gastrointestinal tract or, more likely, within mucosal cells. In a study by Kemmerer and Fraps (1945), rats were fed 9-*cis* βC; at 4 and 6 h after feeding, increased levels of all-*trans* βC and 13-*cis* βC were recovered in the feces, providing evidence of βC isomerization. Tang and Serfaty (1995) evaluated the effects of gastric pH in humans on the isomerization of βC. They provided evidence that the high acidity in the stomach can result in the isomerization of 9-*cis* βC into all-*trans* βC, although the extent of this isomerization in the stomach is unknown.

Because many of the previous studies have used only one source of 9-*cis* βC (Betatene) and only one ratio of all-*trans*/9-*cis* βC, it was the objective of this study to determine if uptake and tissue accumulation of 9-*cis* βC, derived from two algal sources, *Dunaliella baradwil* and *Dunaliella salina*, differed in domestic ferrets.

The data indicate that there is a preferential uptake of all-*trans* over 9-*cis* βC in domestic ferrets. Mucosal cell concentration of βC isomers was evaluated in only one ferret, but segmental analysis (Fig. 2) supports the preferential uptake of all-*trans* over 9-*cis* βC by the intestinal mucosal cells. The ferret used for this study had been supplemented with the Betatene C, which had over twice as much 9-*cis* as all-*trans* βC, but the concentration of all-*trans* βC in the mucosa was ~10-fold higher than the concentration of 9-*cis* βC. The existence of substantial isomerization of 9-*cis* βC to all-*trans* βC before, during and/or after uptake into the mucosal cells cannot be ruled out.

Serum of ferrets fed 9-*cis* βC was essentially free of 9-*cis* βC, which suggests poor absorption of this isomer. However, all of the solid tissues examined in this study contained detectable levels of 9-*cis* βC. The liver had the largest concentration of both all-*trans* and 9-*cis* βC. In the liver, 2.6–8.2% of the administered dose of all-*trans* βC was recovered compared with ~1% of the administered 9-*cis* βC. Thus, the 9-*cis* βC that was absorbed must have been quickly cleared by the liver and other tissues. The adrenal glands had high concentrations of all-*trans* βC, but very little 9-*cis* βC. The kidneys had low amounts of both isomers. All three tissues appeared to have concentrations of all-*trans* βC that increased as the dose

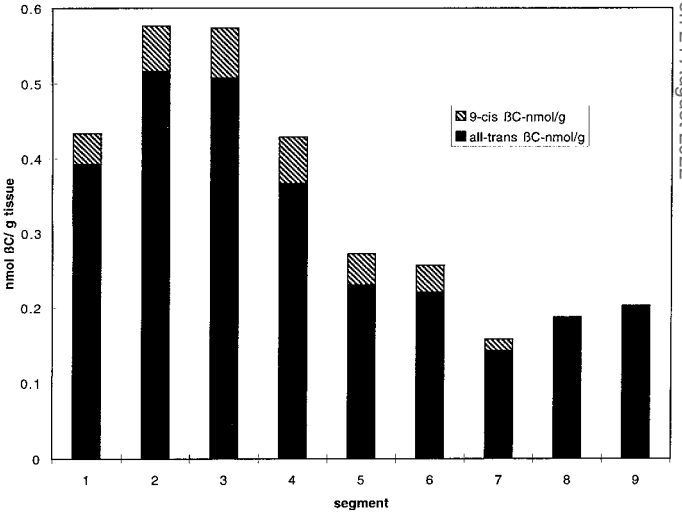

FIGURE 2 The concentration of all-*trans* and 9-*cis* β-carotene (βC) in the mucosal cells from intestinal segments of a ferret supplemented for 9 d with a βC dose consisting of 1.9 μmol all-*trans* and 5.4 μmol 9-*cis* βC.

increased. Only the liver had a high enough concentration of 9-*cis* βC to determine a dose dependency. 9-*cis* βC was detected in tissues of ferrets fed the low all-*trans* βC supplement containing undetectable levels of 9-*cis* βC, suggesting that 9-*cis* βC may be a normal metabolite of all-*trans* βC in tissues.

This laboratory has also evaluated the absorption of 9-*cis* βC in preruminant calves (unpublished data). That species also demonstrated substantial tissue levels, of 9-*cis* βC, particularly in the liver, but an absence of serum 9-*cis* βC after a dose with pure 9-*cis* βC or mixtures of 9-*cis* and all-*trans* βC.

The presence of 9-*cis* βC in the tissues implies that some dietary 9-*cis* βC is absorbed and that the isomer travels through the blood. However, detectable levels of 9-*cis* βC in serum were found only in four of seven ferrets from the Betatene C and two of six ferrets from the *D. bardawil* group. One explanation may be that the liver and other tissues clear the 9-*cis* more quickly than the all-*trans* from the blood. Another explanation may be that the 9-*cis* βC is isomerized to all-*trans* βC before being absorbed, and isomerized back to 9-*cis* βC once in the tissues.

Groups of ferrets were fed doses of 9-*cis* βC from two commercial sources, Betatene and *D. bardawil*, to determine whether the source of 9-*cis* βC affected utilization. There were no differences in tissue concentrations of 9-*cis* βC between the two sources or in the percentage of the dose found in the liver.

In summary, the results of this study suggest the following: *1*) all-*trans* βC is preferentially absorbed compared with 9-*cis* βC; *2*) 9-*cis* βC is not retained in the serum; *3*) there is either preferential clearance of 9-*cis* βC from the blood by liver and other tissues compared with all-*trans* βC and/or isomerization of all-*trans* βC to 9-*cis* βC in the tissues; and *4*) the specific *Dunaliella* source of 9-*cis* βC does not substantially affect its uptake and tissue distribution in domestic ferrets. The results of this study with domestic ferrets support and expand the findings in humans (Jensen et al. 1987, Stahl et al. 1993, Tamai et al. 1995, You et al. 1996) and preruminant calves (unpublished data).

The differential absorption and tissue deposition of 9-*cis* βC compared with all-*trans* βC may not be important metabolically. However, because 9-*cis* βC may be converted to 9-*cis* retinoic acid, limitation of 9-*cis* βC absorption and rapid clearance of 9-*cis* βC from serum may be a regulatory mechanism to reduce the unwanted production of the RXR ligand, 9-*cis* retinoic acid.

LITERATURE CITED

Chandler, L. A. & Schwartz, S. J. (1987) HPLC separation of *cis-trans* carotene isomers in fresh and processed fruits and vegetables. J. Food Sci. 52: 669–672.

Erdman, J. W., Jr., Beirer, T. L. & Gugger, E. T. (1993) Absorption and transport of carotenoids. Ann. N.Y. Acad. Sci. 691: 76–85.

Gaziano, J. M., Johnson, E. J., Russell, R. M., Manson, J. E., Stampfer, M. J., Ridker, P. M., Frei, B., Hennekens, C. H. & Krinsky, N. I. (1995) Discrimination in absorption or transport of β-carotene isomers after oral supplementation with either all-*trans*- or 9-*cis*-β-carotene. Am. J. Clin. Nutr. 61: 1248–1252.

Gugger, E. T., Bierer, T. L., Henze, T. M., White, W. S. & Erdman, J. W., Jr. (1992) β-Carotene uptake and tissue distribution in ferrets (*Mustela putorius furo*). J. Nutr. 122: 115–119.

Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., Stein, R. B., Eichele, G., Evans, R. M. & Thailer, C. (1992) 9-*cis* Retinoic acid is a high affinity ligand for the retinoid X receptor. Cell 68: 397–406.

Jensen, C. D., Howes, T. W., Spiller, G. A., Thomas, S. P., Whittam, J. H. & Scala, J. (1987) Observations on the effects of ingesting *cis*- and *trans*-beta-carotene isomers on human serum concentrations. Nutr. Rep. Int. 35: 413–422.

Kemmerer, A. R. & Fraps, G. S. (1945) The vitamin A activity of neo-β-carotene U and its steric rearrangement in the digestive tract of rats. J. Biol. Chem. 161: 305–309.

Lederman, J. D., Overton, K. M., Hofmann, N. E., Moore, B. J., Thornton, J. & Erdman, J. W., Jr. (1998) Ferrets (*Mustela putorius furo)* are inefficient converters of β-carotene to vitamin A. J. Nutr. 128: 273–279.

Levin, A. A., Sturzenbecker, L. J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzelsen, C., Rosenberger, M., Lovey, A. & Grippo, J. F. (1992) 9-*cis* Retinoic acid stereoisomer binds and activates the nuclear receptor RXR. Nature (Lond.) 355: 359–361.

Nagao, A. & Olson, J. A. (1994) Enzymatic formation of 9-*cis,* 13-*cis,* and all-*trans* retinals from isomers of β-carotene. FASEB J. 8: 968–973.

Ribaya-Mercado, J. D., Holmbren, S. C., Fox, J. G. & Russel, R. M. (1989) Dietary β-carotene absorption and metabolism in ferrets and rats. J. Nutr. 119: 665–668.

Stahl, W., Schwartz, W. & Sies, H. (1993) Human serum concentrations of all-*trans* β- and α-carotene but not 9-*cis* β-carotene increase upon ingestion of a natural isomer mixture obtained from *Dunaliella salina* (Betatene). J. Nutr. 123: 847–851.

Stahl, W., Schwarz, W., Sundquist, A. R. & Sies, H. (1992) *cis-trans* Isomers of lycopene and β-carotene in human serum and tissues. Arch. Biochem. Biophys. 294: 173–177.

Stahl, W. & Sies, H. (1994) Geometrical isomers of β-carotene and lycopene: in vivo studies with humans. In: Retinoids: From Basic Science to Clinical Applications (Livrea, M. A. & Vidali, G., eds.), pp. 29–34. Birkhäuser Verlag, Basel, Switzerland.

Tamai, H., Morinobu, T., Murata, T., Manago, M. & Mino, M. (1995) 9-*cis* β-Carotene in human plasma and blood cells after ingestion of β-carotene. Lipids 30: 493–498.

Tang, G. & Serfaty, C. (1995) Effects of gastric acidity on intestinal absorption of β-carotene in humans. FASEB J. 8: A442 (abs.).

Wang, X.-D., Krinsky, N. I., Benotti, P. N. & Russell, R. M. (1994) Biosynthesis of 9-*cis*- retinoic acid from 9-*cis*-β-carotene in human intestinal mucosa *in vitro*. Arch. Biochem. Biophys. 313: 150–155.

White, W. S., Peck, K. M., Ulman, E. A. & Erdman, J. W., Jr. (1993) The ferret as a model for evaluation of the bioavailabilities of all-*trans*-β-carotene and its isomers. J. Nutr 123: 1129–1139.

You, C., Parker, R. S., Goodman, K. J., Swanson, J. E. & Corso, T. N. (1996) Evidence of *cis-trans* isomerization of 9-*cis* β-carotene during absorption in humans. Am. J. Clin. Nutr. 64: 177–183.

Zhou, J.-R., Gugger, E. T. & Erdman, J.W.J. (1996) The crystalline form of carotenes and the food matrix in carrot root decrease the relative bioavailability of beta- and alpha-carotene in the ferret model. J. Am. Coll. Nutr. 15: 84–91.