All-*trans* β-Carotene Appears to Be More Bioavailable than 9-*cis* or 13-*cis* β-Carotene in Gerbils Given Single Oral Doses of Each Isomer[1,2]

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ABSTRACT Male gerbils (28 d old) were used to investigate the β-carotene (βC) isomer pattern in the intestine and tissues 6 h after ingestion of three βC isomers. After a 49- to 52-d period of consuming the AIN93G diet without vitamin A (VA) or βC, three groups ($n = 7$) were gavaged with crystalline all-*trans* (at)βC, 9-*cis* (9c)βC or 13-*cis* (13c)βC solubilized in oil and a control group ($n = 5$) with oil alone. Total βC per dose for gerbils in the atβC, 9cβC and 13cβC groups was 384 ± 3, 391 ± 2 and 386 ± 2 nmol, respectively. After 6 h, gerbils were killed and serum, stomach contents, small intestinal contents (SIC), small intestinal mucosal scrapings (SIM) and liver were collected. βC and VA in tissues were quantified using HPLC. Nonspecific isomerization of βC occurred in the digestive tracts of gerbils administered βC; the greatest effect was in the SIC of the 13cβC (50:50 *cis*:*trans*) and 9cβC (70:30 *cis*:*trans*) groups. Concentrations of total βC in the SIM of gerbils administered at βC were greater than those intubated with 9cβC and 13cβC ($P < 0.05$). Gerbils that received atβC had greater total βC concentrations in serum ($P < 0.05$) and total βC stores in liver ($P < 0.01$) compared with those administered 9cβC and 13cβC. Gerbils intubated with 9cβC had higher levels of total βC in serum ($P = 0.05$) and liver ($P < 0.01$) compared with those intubated with 13cβC. Because of its preferential uptake, transport and tissue accumulation, atβC appears to be a more bioavailable isomer than 9cβC or 13cβC in gerbils. J. Nutr. 132: 2700–2708, 2002.

KEY WORDS: • *all-trans* β-carotene • *9-cis* β-carotene • *13-cis* β-carotene • *vitamin A* • *gerbils*

Although all-*trans* β-carotene (atβC)[3] is the predominant isomer in many fresh fruits and vegetables, thermal processing can substantially increase the proportions of geometrical *cis* isomers of βC in these foods, in particular 13-*cis* (13*c*) and 9-*cis* (9*c*) βC (1–4). Human serum contains largely atβC with only small or negligible amounts of 13cβC and 9cβC (5–10) after the ingestion of βC isomers. However, considerable amounts of *cis* isomers of βC are present in various human (11,12) and animal tissues (13–17). These observations support the suggestion that βC isomers may possess isomer-specific biological actions (6). All-*trans* and 9-*cis* βC, in particular, have received the most attention because they can be metabolized to their respective retinoic acid isomers, all-*trans* retinoic acid and 9-*cis* retinoic acid (18,19), both of which are active in gene regulation (20,21).

Consumption of *cis* isomers of βC from foods may be substantial, but their bioavailability is poorly understood. Results from human studies consistently report elevated serum/plasma responses after ingestion of atβC, whereas the 9cβC response is negligible even after ingestion of large amounts of this isomer (5–9). A similar trend was reported in chylomicrons of humans (22), suggesting a preferential enrichment of the all-*trans* isomer in the small intestinal mucosa. There is also some plausible evidence that βC isomerizes at some point during the digestive, uptake or absorption processes (23,24), and that the rates of cleavage of βC isomers to vitamin A (VA) and the composition of the respective isomer metabolites vary (18,25). Despite low serum and chylomicron concentrations, 9cβC is present in substantial quantities in liver tissue from humans (12); it accumulates in liver tissue of chicks (14), rats (15) and ferrets (17) after ingestion of mixtures of atβC and 9cβC. The 9-*cis* and 13-*cis* βC isomers also appear to be less efficient precursors of liver retinol than the all-*trans* isomer (26,27). The contrasting serum responses and tissue accumulation of βC isomers and their metabolites may be explained by differences in intestinal absorption, conversion to VA, rates of uptake of βC from the circulation by tissues and/or isomerization in tissues.

The objective of this study was to investigate some of these factors by comparing the relative bioavailabilities of atβC, 9cβC and 13cβC in gerbils 6 h after an oral dose of each isomer in oil. Because gerbils absorb βC intact at physiologic doses (28), it is possible to determine tissue levels of βC and VA in a relatively short time period after a single dose. We examined events in the early stages of digestion by comparing the βC isomer composition of the purified dose to that in the residual contents of the stomach and the small intestine. We also estimated potential differences in the uptake of the βC

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[3] Abbreviations used: atβC, all-*trans* β-carotene; βC, β-carotene; 9cβC, 9-*cis* β-carotene; 13cβC, 13-*cis* β-carotene; VA, vitamin A.

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isomers by quantifying their levels in mucosal scrapings from the small intestine. Serum responses and tissue accumulation of βC isomers were evaluated to explore whether differences in tissue uptake are determinants of serum responses. Finally, we quantified total VA in liver to evaluate the value of each isomer as a precursor of VA.

MATERIALS AND METHODS

Chemicals. Standards of crystalline βC isomers (a generous gift from Dr. Regina Goralczyk, Hoffmann-La Roche, Basel, Switzerland) were evaluated photometrically after dissolution in hexane. Concentrations were calculated using E(1%, 1 cm) = 2590 at 450 nm for at*β*C, E(1%, 1 cm) = 2550 at 445 nm for 9*cβ*C, and E(1%, 1 cm) = 2090 at 443 nm for 13*cβ*C (29). Echinenone (gift from Hoffmann La-Roche), was also dissolved in hexane and used as an internal standard for quantification of βC isomers. Standards of all-*trans* retinol [E(1%, 1 cm) = 1850 in ethanol at 325 nm, Sigma Chemical, St. Louis, MO], 13-*cis* retinol (Sigma Chemical, St. Louis, MO) and 9-*cis* retinol (Hoffmann-La Roche) were dissolved in ethanol. The purity of all standards was verified using HPLC (at*β*C and 9*cβ*C were ~99–100%; 13*cβ*C was 95% with impurities of at*β*C; all-*trans* retinol was ~99–100%).

Animals, diet and study design. Male Mongolian gerbils (n = 26; *Meriones unguiculatus*), 28 d of age, with a body weight of 30 ± 2 g were obtained from Charles River Laboratories (Raleigh, NC). Upon arrival, gerbils were individually housed in plastic shoebox cages and given free access to water and a pelleted depletion diet, i.e., AIN93G diet (30) formulated with vitamin-free casein, cottonseed oil and without any known source of VA or βC for 49–52 d. The duration of the depletion period was based upon the amount of time required to reduce initial liver VA status, which was estimated to be 60 d according to Thatcher and co-workers (31). The goal for this depletion period was to reduce liver VA stores in gerbils to a "marginally sufficient" status (≥ 20 μg/g or 70 nmol/g liver). The advantages of a depletion period include decreasing experimental variance by equalizing liver VA levels before treatment and increasing the ability to detect treatment effects by reducing liver VA levels to a "marginally sufficient" status. The overall health of the gerbils was monitored daily and body weight was recorded once each week. Room temperature was constant and lighting was provided on a diurnal cycle of 12-h light:12-h dark for the duration of the study. The University of Illinois Laboratory Animal Care Advisory Committee approved all animal handling procedures.

After depletion, the gerbils were assigned to one of four treatment groups, and subsequently repleted with a single, oral dose of cottonseed oil with or without βC. Three groups of gerbils (n = 7) received at*β*C, 9*cβ*C or 13*cβ*C in oil by gastric intubation. We calculated a βC dose of ~387 nmol on the basis of the daily requirement of VA from the full formula AIN93G diet, assuming an intake of 6–10 g food/(d · 100 g body) and between 6:1 and 13:1 molar conversion efficiency of βC isomer to VA. The gerbils in the control group (n = 5) received a dose of oil without βC. Each gerbil was anesthetized before gastric intubation.

Briefly, the gerbil was placed in an induction chamber connected to a precision vaporizer, and isoflurane (2.5–3%) was administered with oxygen (1.5%) by inhalation until loss of consciousness. The oil dose was administered using a 1-mL syringe inserted into a stainless steel gastric feeding needle (20-gauge × 3.8 cm) with a ball tip (1 mm). The gerbil was then returned to its cage and regained consciousness within 15–30 s. The syringe was weighed before and after gavage to determine the actual oil dose delivered to each gerbil. In a pilot study, we observed that gerbils practiced coprophagy to a greater extent when deprived of food overnight and after treatment (unpublished). Thus, in this study, gerbils were given free access to water and food before and after treatment, which substantially decreased the amount of feces and food observed in the upper gastrointestinal tract at the time of killing.

After dosing (6 h), the gerbils were anesthetized by CO_2, and blood was collected immediately by cardiac puncture, followed by cervical dislocation. We chose the 6-h time point on the basis of the amount of time required to simultaneously optimize serum and liver responses of βC after a single oral dose of at*β*C according to Pollack and co-workers (28). Blood samples were centrifuged for 10 min at 2400 × g at 4°C and serum was collected. Samples of residual stomach contents and small intestinal contents were removed from each gerbil. The small intestine was removed and thoroughly rinsed with ice-cold, 9 g/L NaCl solution. A 15-cm section of small intestine was sliced longitudinally and mucosal cells were collected by scraping the tissue with a glass slide. Liver, lungs, adrenal glands, kidneys and spleen were removed from all gerbils, rinsed with saline, dried and weighed. All tissues were snap-frozen in liquid nitrogen, stored at −80°C and analyzed within 4 mo. A partial dose recovery from the upper gastrointestinal tract was performed in two gerbils from each treatment group on two separate days. Hemostats were used to clamp off the stomach and small intestine to prevent loss of contents before excision. Each of these tissues were then excised, rinsed twice with 20 mL ice-cold 9 g/L NaCl solution into preweighed plastic bags, stored in ice at 4–5°C and analyzed within 24–48 h. Experimental procedures were performed in 4 d. Each treatment was represented across the 4 d to avoid any bias.

Oil dose preparation and analyses. Crystals of at*β*C, 9*cβ*C and 13*cβ*C were solubilized in equal amounts of cottonseed oil. Briefly, stock solutions of each βC isomer were prepared by dissolution in hexane (100 mg βC/L). Specific volumes of the stock solution were then pipetted into an amber-colored vial containing an appropriate volume of oil and placed under argon to evaporate the hexane. Oils were mixed by hand for ~30 s between additions of stock solution. Complete evaporation of hexane was confirmed gravimetrically, and solubility of βC in oil was confirmed by light microscopy using 100X magnification.

Preliminary storage studies of the oils were conducted at ambient temperature in our laboratory to monitor recrystallization of βC in oil. Crystals were first visible only in the at*β*C oil on d 3 of storage, whereas no crystals were visible in the 9*cβ*C or 13*cβ*C oils at that time. Therefore, βC isomer oils were prepared every 2 d during the 4-d dosing period. The purity of the βC isomer oils and the molar concentration of the βC isomer in the oils were evaluated by HPLC using the method of Yeum et al. (32). Duplicate aliquots of each βC isomer oil (~225 mg) were extracted with hexane (1 mL) without saponification. A 20-μL portion of the hexane solution was collected and evaporated using an Automatic Speed Vacuum System, Model AES1010 (Savant Instruments, Farmington, NY). Extracts were flushed with argon and analyzed immediately.

Tissue extraction procedures. The stomach contents, small intestinal contents, serum samples (300–800 μL) and mucosal scrapings of the small intestine were mixed with absolute ethanol containing 1 g/L BHT and extracted three times with excess hexane without saponification. Liver tissue was extracted in duplicate. Tissue samples (200–250 mg) were minced and mixed with 4 mL of BHT/ethanol to denature protein, and were saponified with 1 mL potassium hydroxide (500 g/L) in a water bath at 60°C for 25 min. Samples were immediately placed in ice and cooled to ambient temperature. Distilled water (3 mL) was added and this mixture was extracted three times with hexane (8 mL). Hexane layers were combined and evaporated as described above. Dried extracts were flushed with argon and stored at −20°C. A separate extraction of liver tissue was completed to evaluate liver retinol isomers. Extractions of the lungs, adrenal glands, kidneys and spleen were performed on groups of pooled tissues (n = tissues from 2 gerbils/group) from each treatment group. Each group consisted of tissue from the same two gerbils across tissue analysis. Pooled tissues were minced and extracted as described for liver tissue. Samples of the pelleted diet were also extracted in triplicate by methods described previously (33). Analysis of retinol using HPLC was completed immediately after extraction, and that of βC within 24 h of extraction.

The potential effects of extraction procedures on isomerization of βC and retinol were considered and minimized by performing all procedures under yellow lighting, using BHT in solvents and securing test tubes in ice immediately after saponification and during hexane extraction. We did not observe extensive isomerization of βC in tissues with the mild heating conditions of saponification (20–30 min, 60°C) used in our laboratory. In addition, carotenoid standards remained stable in our laboratory when dissolved in hexane and

stored in amber glass vials flushed with argon for up to 2 wk at −20°C.

HPLC analyses. β-Carotene isomers in the oils and tissues were separated using a 250 mm × 10 mm YMC C30 column (YMC, Wilmington, NC) at 450 nm according to the gradient, reverse-phase HPLC method of Yeum and co-workers (32). Individual isomers of βC from tissues were identified using retention times of standards (13*c*βC at 13.7 min, a*t*βC at 15.3 min, 9*c*βC at 15.9 min) and quantified relative to echinenone (internal standard) by determining peak areas calibrated against known amounts of the standards.

Retinol was separated isocratically using a Supelco LC-18 column (#58298 Supelco, Bellefonte, PA) and a mobile phase of methanol/acetonitrile/chloroform (47:47:6, v/v/v) at a flow rate of 1.5 mL/min with detection at 325 nm. Retinol was identified by retention time of the all-*trans* retinol standard (3.9 min) and quantified by determining peak areas calibrated against known amounts of this standard. Retinol isomers in liver tissue were evaluated according to the method of MacCrehan and Schonberger (34). Briefly, retinol isomers were separated using a reverse-phase Vydac C18 column [Vydac 201 TP 54, 25 cm × 4.6 mm (i.d.); The Separations Group, Hesparia, CA] with an isocratic mobile phase of methanol/butanol/water (10 mmol/L ammonium acetate) (65:25:10, v/v/v) at 1 mL per min under detection at 325 nm. Retinol isomers in liver were identified using retention times of the standards (9*c*-retinol at 15.3 min, 13*c*-retinol at 16.0 min, a*t*-retinol at 17.7 min).

The HPLC system for all analyses was comprised of a Dynamax Model SD200 Solvent Delivery System, a Dynamax Absorbance Detector UV D-I, and a Dynamax HPLC Methods Manager integrator (Rainin Instrument, Woburn, MA). The analytical column for each method was protected by a precolumn (Upchurch Scientific, Oak Harbor, WA) packed with ODS C-18 (Alltech Associates, Deerfield, IL). All samples were injected manually. Standards of βC isomers and retinol isomers were injected periodically throughout the day of analysis to establish retention times of those compounds from tissues.

Statistical analyses. Data analyses were completed using the Statistical Analysis System (SAS, 6.12, Institute, Cary, NC). The proportions of total βC *cis* isomers in the oil doses, stomach contents, small intestinal contents, mucosal scrapings from the small intestine, serum and liver were analyzed using one-way ANOVA. If the *F*-statistic for the ANOVA was significant, means were further evaluated by least-square difference (LSD). The quantifiable levels of total βC and retinol in the oil doses and tissues were analyzed using *1*) one-way ANOVA and orthogonal contrast coding to test the effect of βC isomer on levels of total βC and total retinol in tissues, and *2*) one-way ANOVA and LSD. Significance of differences was determined using a P-value < 0.05. All values are represented by means ± SEM.

RESULTS

Body weight and diet. The body weight of gerbils more than doubled from 30 ± 2 g to 67 ± 2 g during the 49- to 52-d depletion period. Liver VA of gerbils in the control group after VA depletion [10 ± 3 μg/g (34 ± 9 nmol/g liver)] was lower than we predicted [≥ 20 μg/g (70 nmol/g liver)]. HPLC analysis of the depletion diet revealed that it contained a small amount of vitamin A [0.5 ± 0.2 μg/g diet (1.7 ± 0.1 nmol/g diet)] and trace levels of βC.

βC isomer oils. The dose of oil (185 ± 2 mg oil) and the total βC in the oils did not differ. The purity of βC isomers in the respective oils was relatively high compared with the crystalline isomer, indicating that isomerization was minimized during the oil preparation procedures. The a*t*βC oil (97.8 ± 0.4% a*t*βC, 1.2 ± 0.4% 9*c*βC, 1.0 ± 0.1% 13*c*βC) contained 2075 ± 7 nmol total βC/g oil or 384 ± 3 nmol total βC/dose of oil. The 9*c*βC oils (1.8 ± 0.6% a*t*βC, 98.7 ± 0.6% 9*c*βC, 0% 13*c*βC) contained 2116 ± 5 nmol total βC/g oil or 391 ± 2 nmol total βC/dose of oil. The 13*c*βC oil (8.0 ± 0.9% a*t*βC, 0% 9*c*βC, 92.0 ± 0.9% 13*c*βC) contained 2085 ± 6 nmol total βC/g oil or 386 ± 2 nmol total βC/dose of oil.

β-Carotene isomer profiles in contents of the digestive tract. The proportion of total βC as *cis* isomers in the oil dose, the residual contents of the stomach and the small intestine, the mucosal scrapings from the small intestine, the serum and the liver collected from gerbils in each βC isomer group is illustrated in **Figure 1**.

The dose administered to the a*t*βC group (97.8 a*t*βC) contained 2.2% *cis*-βC (Fig. 1A). The residual contents of the stomach from this group contained a significantly higher proportion of *cis* βC (21 ± 3%) with the same proportion in the residual contents of the small intestine (22 ± 3%). However, the small intestinal mucosal scrapings contained a significantly higher proportion of *cis* βC (34 ± 1%) than these two fractions. In addition, the 13-*cis* isomer represented most of the total *cis* βC contained in these fractions. Interestingly, the proportions of *cis* βC in serum (13 ± 2%) and liver (14 ± 1%) were significantly lower than that in the other fractions and were closest in *cis* βC content to the dose.

The dose given to the 9*c*βC group contained 98.7% *cis* βC (Fig. 1*B*). A progressive and significantly lower proportion of *cis* βC was observed in the residual stomach contents (89 ± 3% *cis* βC) and in the contents of the residual small intestine (69 ± 4% *cis* βC). However, the scrapings from the small intestine contained a significantly higher proportion of *cis* βC (80 ± 2% *cis* βC) than that in the residual contents of the small intestine. The proportion of total *cis* βC in serum (43 ± 2%) was significantly lower than that observed in any other fraction. In contrast, the proportion of *cis* βC in liver (88 ± 3%) was high and not different from that in the residual stomach contents. Most of the total *cis* βC in all fractions was the 9-*cis* isomer.

The extent of isomerization appeared to be greatest in the tissue fractions from the 13*c*βC group. The oil dose administered to this group contained 92.0% *cis* βC (Fig. 1C). The residual contents of the stomach from this group contained a significantly lower proportion of *cis* βC (60 ± 3%). The residual contents of the small intestine (48 ± 5% *cis* βC) and of the mucosal scrapings from the small intestine (39 ± 2%) were lower than in the stomach. Most of the total *cis* βC contained in these fractions was the 13-*cis* isomer. As in serum from the 9*c*βC group, the proportion of *cis* βC in serum from the 13*c*βC group (18 ± 7%) was significantly lower than that in any other fraction. In addition, the liver contained a significantly higher proportion of *cis* βC (38 ± 2%) than in serum, but in this case, the proportion was not different from that observed in the mucosal scrapings.

The differences in extents of isomerization in the residual contents of the stomach and small intestine among the βC isomer oils were investigated further by a quantitative recovery of βC in the upper gastrointestinal tract of gerbils ($n = 2$) 6 h after the oil was gavaged (**Table 1**). The total amount of βC in the residual contents of the stomach and the small intestine represents a portion of the amount not taken up. The recovery of βC from the dose after 6 h was low (a*t*βC group, 4.1%; 9*c*βC group, 0.6%; 13*c*βC group, 1.8%), and most of the βC was present in the contents of the stomach. We visually observed substantial quantities of oil in the large intestines from gerbils in all groups, suggesting that a large portion of the oil had passed through the small intestine within 6 h.

β-Carotene and VA in mucosal scrapings from the small intestine, serum and liver. Quantitative levels of total βC in the mucosal scrapings from the small intestine in serum and in liver of all groups are presented in **Figure 2** and **Table 2**. All βC isomer-treated groups had significantly more total βC in these three tissues than the control group. Although a*t*βC was the primary isomer in tissues of gerbils intubated with a*t*βC

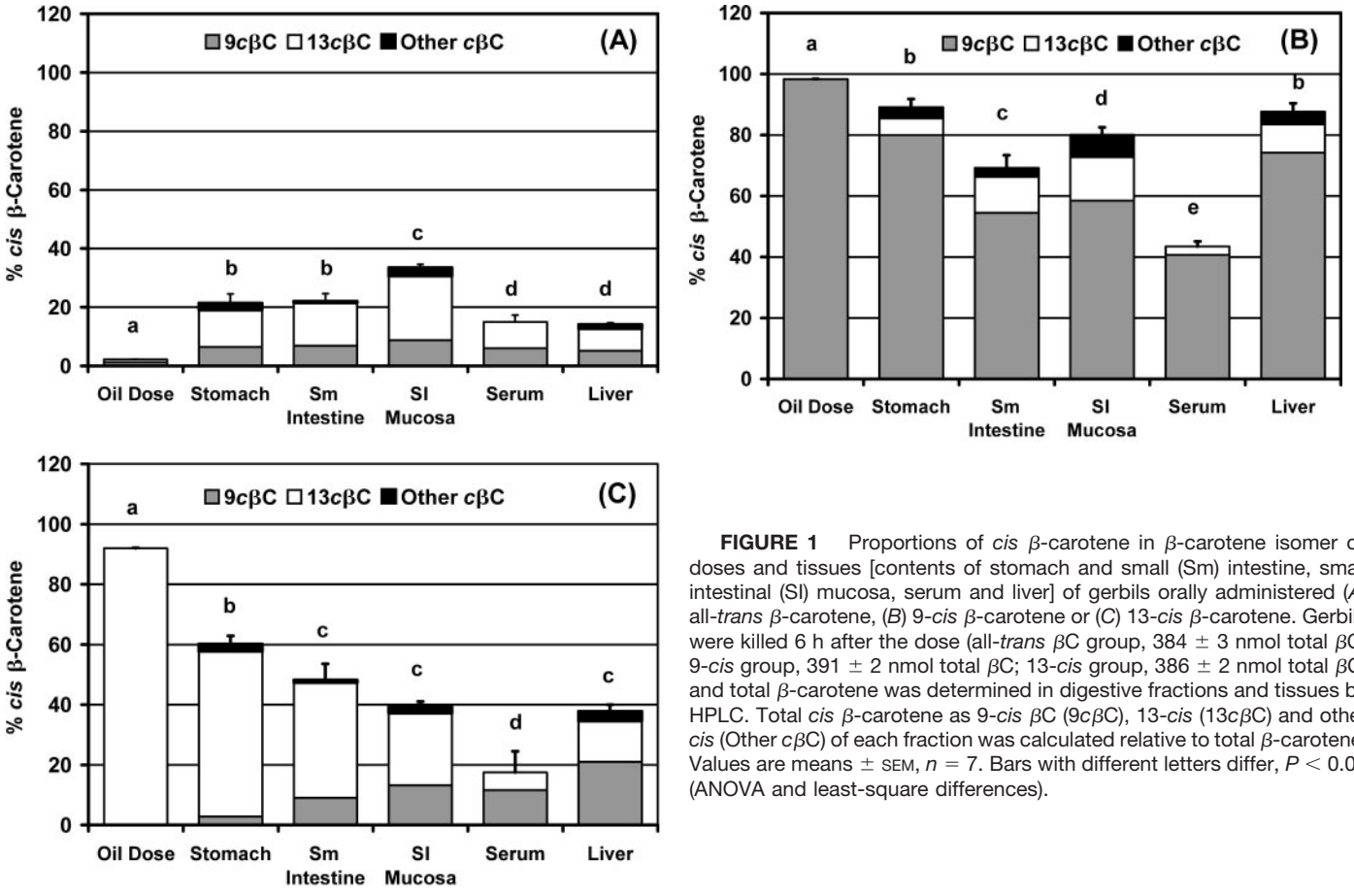

FIGURE 1 Proportions of *cis* β-carotene in β-carotene isomer oil doses and tissues [contents of stomach and small (Sm) intestine, small intestinal (SI) mucosa, serum and liver] of gerbils orally administered (*A*) all-*trans* β-carotene, (*B*) 9-*cis* β-carotene or (*C*) 13-*cis* β-carotene. Gerbils were killed 6 h after the dose (all-*trans* βC group, 384 ± 3 nmol total βC; 9-*cis* group, 391 ± 2 nmol total βC; 13-*cis* group, 386 ± 2 nmol total βC) and total β-carotene was determined in digestive fractions and tissues by HPLC. Total *cis* β-carotene as 9-*cis* βC (9cβC), 13-*cis* (13cβC) and other *cis* (Other cβC) of each fraction was calculated relative to total β-carotene. Values are means ± SEM, $n = 7$. Bars with different letters differ, $P < 0.05$ (ANOVA and least-square differences).

(Fig. 2A) and 9cβC was the primary isomer in tissues of gerbils given 9cβC (except serum) (Fig. 2B), the same trend was not observed for gerbils administered 13cβC (Fig. 2C). Tissues from these gerbils contained > 50% atβC.

β-Carotene quantified in the mucosal scrapings from the small intestine from each group is shown in Table 2. Low levels of βC were detected in the mucosal scrapings from the control group. The atβC group had higher levels of total βC than the 9cβC group and the 13cβC group ($P = 0.03$). Total βC levels in the 9cβC group were also higher than those in the 13cβC group.

Table 2 also shows the βC in serum from each group. βC was not detected in serum from the control group. Serum βC concentrations in the atβC group were greater than those in the 9cβC and the 13cβC groups ($P = 0.04$). Although the 9cβC group had significantly higher serum βC than the 13cβC group, serum βC levels from both *cis*-treated groups were lower than levels in the atβC group and in some cases almost undetectable. Notably, the primary βC isomer in serum of both *cis* groups was the all-*trans* (Fig. 2B, C).

Total βC quantified in liver from each group is shown in Table 2. Although endogenous levels of βC were quantified in the livers of the control group, total βC was ~11-, 4- and 1-fold higher in livers from the atβC, 9cβC and 13βC groups, respectively. The primary βC isomers in livers from the atβC, 9cβC and 13cβC groups were the all-*trans*, the 9-*cis* and the all-*trans*, respectively.

VA levels in serum and liver from each oil group are shown in **Table 3**. The liver VA stores in all three βC isomer-treated groups tended to be higher than the control group ($P = 0.063$) and did not differ from one another. Additional HPLC analyses revealed the presence of all-*trans*, 9-*cis* and 13-*cis* retinol in livers from all groups, and interestingly, the composition of retinol isomers did not differ among the groups (**Fig. 3**). The isomeric composition of liver retinol for the control group was 90 ± 2% at-retinol, 7 ± 1% 13c-retinol and 3 ± 2% 9c-retinol and for the atβC group, 92 ± 2% at-retinol, 6 ± 1% 13c-retinol and 2 ± 2% 9c-retinol. Proportions of liver retinol isomers for the 9cβC group were 87 ± 4% at-retinol, 8 ± 2% 13c-retinol and 5 ± 3% 9c-retinol, and for the 13cβC group, 91 ± 1% at-retinol, 7 ± 1% 13c-retinol and 2 ± 1% 9c-retinol.

β-Carotene and VA in lung, adrenal, spleen and kidney. Concentrations of βC in lung, adrenal, spleen, and kidney from each group are shown in Table 2. βC was not detected in these tissues from the control group. Levels of total βC in lung, adrenal, spleen and kidney from the atβC group were dramatically and significantly higher than those from the 9cβC and 13cβC groups. βC in lung from the atβC group was 64% all-*trans* and 36% 9-*cis* βC (data not shown). Adrenal glands contained 79% atβC and 21% *cis*-βC, of which 15% was 13cβC. Spleen tissue from the atβC group contained 94% atβC and 6% 9cβC. Levels of βC in kidney from the atβC group were lower than those in other tissues from this group. The isomer profile in kidney was 50% atβC and 50% *cis*-βC, of which 51% was the 13c isomer.

Levels of βC in lung were higher in the 13cβC group than the 9cβC group ($P = 0.04$). βC in lung from the 9cβC group was 54% all-*trans* and 46% 9-*cis* βC, and that from the 13cβC group was 24% all-*trans* and 76% *cis* βC, of which 67% was 13cβC. In contrast to lung, levels of βC in spleen were higher in the 9cβC group than the 13cβC group ($P = 0.001$). Spleen

TABLE 1

Quantitative recovery of cis and trans β-carotene (βC) 6 h after oral doses of all-trans, 9-cis, or 13-cis β-carotene in gerbils[1,2]

All-*trans* β-carotene oil		Total βC dose recovery = 4.1%		
Fraction	*trans* βC	*cis* βC	Total βC	*cis* βC
	nmol			%
Oil dose	371.4	8.5	379.9	2.2
Stomach contents	9.3	2.0	11.3	17.7
SI Contents	2.1	1.5	3.6	41.7
SI Mucosa	0.54	0.27	0.81	33.3
9-*cis* β-carotene oil		Total βC dose recovery = 0.6%		
Fraction	*trans* βC	*cis* βC	Total βC	*cis* βC
	nmol			%
Oil dose	7.0	390.6	397.6	98.2
Stomach contents	0.3	1.6	1.9	84.2
SI Contents	0.04	0.08	0.12	66.7
SI Mucosa	0.12	0.34	0.46	73.9
13-*cis* β-carotene oil		Total βC dose recovery = 1.8%		
Fraction	*trans* βC	*cis* βC	Total βC	*cis* βC
	nmol			%
Oil dose	31.2	360.1	391.3	92.0
Stomach contents	2.2	4.2	6.4	65.6
SI Contents	0.2	0.2	0.4	50.0
SI Mucosa	0.11	0.06	0.17	35.2

[1] Data represent the recovery of a dose of oil containing all-*trans* βC (384 ± 3 nmol total βC), 9-*cis* βC (391 ± 2 nmol total βC) and 13-*cis* βC (386 ± 2 nmol total βC) fed orally to groups of gerbils by gastric intubation. Stomach contents, small intestinal (SI) contents, and SI mucosa were removed and analyzed for total βC using HPLC.

[2] Data means of two gerbils. *cis* βC was calculated as a percentage of total βC from each fraction.

from the 9*c*βC group contained 31% at*β*C and 69% *cis*-βC, all of which was 9*c*βC. βC in spleen from the 13*c*βC group was 100% all-*trans* βC. Adrenal gland from the 9*c*βC group contained 14% at*β*C and 86% 9*c*βC, and that from the 13*c*βC group contained 100% at*β*C. The composition of βC isomers in kidney from the 9*c*βC group was 41% at*β*C and 59% 9*c*βC. βC was not detected in kidney from the 13*c*βC group.

VA concentrations in lung, adrenal, spleen and kidney from each group are shown in Table 3. There were no differences in VA in any of these tissues except for kidney. However, although VA levels were quantifiable in this tissue, they were extremely low compared with other tissues.

DISCUSSION

Cis isomers of β-carotene (βC) including 13-*cis* (13*c*) and 9-*cis* (9*c*) are present in substantial amounts in processed foods and thus can be an important source of dietary βC (1–4). However, little is known about the bioavailability of *cis* isomers of βC. Elevated levels of all-*trans* (at) βC and 13*c*βC in human serum have been reported in response to dietary and supplemental intake of at*β*C, but the normally low serum levels of 9*c*βC are not increased, even after high doses of the 9-*cis* isomer. Yet, relatively high levels of 9*c*βC have been reported in tissues of humans (11,12) and several species of animals (14–17). In rats, 9-*cis* and 13-*cis* βC also appear to be less efficient precursors of liver VA than the all-*trans* isomer (26,27). It is not clear whether this anomaly surrounding 9*c*βC bioavailability is because of in vivo isomerization, less efficient uptake into the small intestine, a difference in conversion to VA, and/or more rapid tissue uptake after absorption.

We explored these factors by evaluating the relative bioavailability of at*β*C, 9*c*βC and 13*c*βc in gerbils 6 h after a single dose of each isomer solubilized in cottonseed oil. We chose gerbils to study βC bioavailability because of their ability to absorb βC intact at physiologic doses (28) and to convert βC to VA with an efficiency similar to that of humans (35). Dietary βC also reverses marginal VA status in gerbils (31,35). Therefore, we expected to observe serum and tissue responses to a dose of at*β*C and 9*c*βC similar to those in humans.

The growth rate of gerbils in our study was not different from those observed by House and co-workers (36) who reported no difference in growth between gerbils fed the AIN93G diet and those fed a commercial pelleted rodent diet (Prolab RMH 1000 Rodent Diet, Agway, Syracuse, NY). Liver VA of gerbils in the control group after 49–52 d of VA depletion was lower than we predicted, i.e., 10 ± 3 μg/g (34 ± 9 nmol/g liver). An adult human with a liver vitamin A concentration of < 10 μg/g is considered to have a poor-to-marginal vitamin A status (37). However, according to Olson (38), individuals in the marginal state do not show clinical signs of deficiency but may have impaired physiologic responses. This may have been the case with the gerbils in our study because they did not exhibit ill health or any physical signs of compromised VA status.

Initially, we were interested in exploring whether the differences in bioavailability of at*β*C, 9*c*βC and 13*c*βC were due in part to steric rearrangement or isomerization of βC isomers in the digestive tract. Kemmerer and Fraps (23) observed a progressive increase in nonspecific isomerization of 9*c*βC in the digestive tract and feces of rats 4 and 6 h after feeding 9*c*βC solubilized in oil. Substantial nonspecific isomerization has also been reported in the gut washings and feces of chicks 4 h after a dose of different carotenoids dissolved in oil and incorporated into a diet (39). It has been suggested that βC isomerization in the digestive tract may be because of heat, gastric pH and/or intestinal microflora (40).

Similar to the observations of Kemmerer and Fraps (23), nonspecific isomerization of βC occurred in the digestive tract of gerbils in each βC isomer group 6 h after the oil dose. Although significant changes in the proportions of *cis* βC were observed in the residual stomach contents relative to the dose in all three βC groups, additional isomerization in the small intestine occurred only in gerbils administered the *cis* βC oils. Moreover, the extent of isomerization appeared to be higher in those gerbils gavaged with the 13*c*βC oil than those administered the 9*c*βC oil. The βC composition in the contents of the small intestine was approximately a 50:50 and a 70:30 ratio of *cis*:*trans* βC for gerbils given the 13*c*βC and the 9*c*βC oils, respectively. These ratios are in striking contrast to the 20:80 ratio of *cis*:*trans* βC in the contents of the small intestine of gerbils administered the at*β*C oil. In quantitative kinetic studies using ^{1}H NMR, Doering and co-workers (41) suggested that the rate of *cis*-to-*trans* isomerization decreases progressively as the location of the *cis* bond departs from the central position, which could explain the higher proportion of *trans* βC in the small intestinal contents from gerbils gavaged with the 13*c*βC oil compared with those given the 9*c*βC oil.

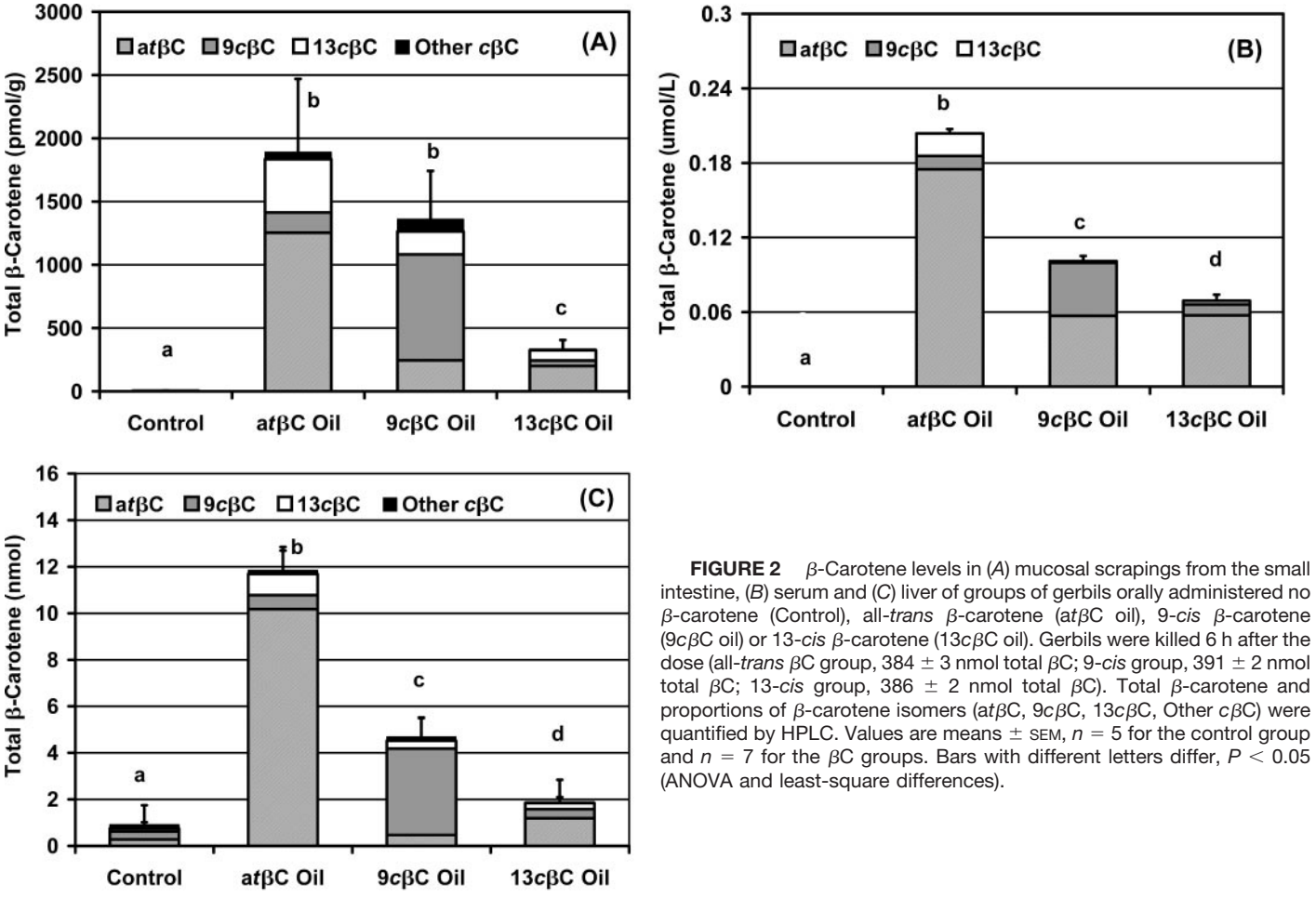

FIGURE 2 β-Carotene levels in (*A*) mucosal scrapings from the small intestine, (*B*) serum and (*C*) liver of groups of gerbils orally administered no β-carotene (Control), all-*trans* β-carotene (atβC oil), 9-*cis* β-carotene (9cβC oil) or 13-*cis* β-carotene (13cβC oil). Gerbils were killed 6 h after the dose (all-*trans* βC group, 384 ± 3 nmol total βC; 9-*cis* group, 391 ± 2 nmol total βC; 13-*cis* group, 386 ± 2 nmol total βC). Total β-carotene and proportions of β-carotene isomers (atβC, 9cβC, 13cβC, Other cβC) were quantified by HPLC. Values are means ± SEM, $n = 5$ for the control group and $n = 7$ for the βC groups. Bars with different letters differ, $P < 0.05$ (ANOVA and least-square differences).

TABLE 2

β-Carotene (βC) levels in intestinal mucosal scrapings, serum, liver, lungs, adrenals, spleen, and kidneys from gerbils 6 h after oral doses of all-trans-βC, 9-cis-βC, or 13-cis-βC in cottonseed oil[1]

	Mucosa	Serum	Liver	Lung	Adrenal	Spleen	Kidney
	pmol/g	*μmol/L*	*nmol*	*pmol/g*			
Treatment[2]							
Control	8 ± 5	ND	0.8 ± 0.1	ND	ND	ND	ND
All-*trans* βC	1892 ± 578	0.211 ± 0.04	12 ± 1	50 ± 1.2	941 ± 51	1020 ± 56	71 ± 16
9-*cis*-βC	1362 ± 381	0.100 ± 0.04	5 ± 0.8	21 ± 1.2	286 ± 17	679 ± 33	14 ± 0.3
13-*cis*-βC	337 ± 70	0.069 ± 0.03	2 ± 0.2	27 ± 2.5	55 ± 0.9	221 ± 14	ND
Statistics[3]							
βC vs. Control	0.0211	0.0036	<0.0001	<0.0001	0.0038	0.0004	0.0026
cis vs. *trans*	0.0256	0.0372	<0.0001	<0.0001	0.0001	0.0003	0.0376
13 *cis* vs. 9 *cis*	0.0587	0.0470	0.0030	0.0374	NS	0.0010	—

[1] βC was quantified using HPLC. For tissues in all of the βC groups and for mucosa and liver in the control group, values represent means ± SEM; for serum, $n = 5$ for the all-*trans* βC group and $n = 3$ for the 9-*cis* βC and 13-*cis* βC groups; for liver and mucosa, $n = 5$ for the control group and $n = 7$ for the βC groups; for lung, adrenal, spleen, and kidney, $n = 3$ (pools of tissues from 2 gerbils). Values for the control groups for serum, lung, adrenal, spleen, and kidney represent averages of $n = 2$ (pools of tissues from 2 gerbils); ND, not detected.

[2] Oil dose = 185 ± 2 mg (mean ± SEM); Control = cottonseed oil alone, all-*trans* βC oil contained 384 ± 3 nmol total βC/dose, 9-*cis* βC oil contained 391 ± 2 nmol total βC/dose, and 13-*cis* βC oil contained 386 ± 2 nmol total βC/dose.

[3] ANOVA with orthogonal coding; βC vs. Control = all βC groups compared with the Control group; *cis* vs. *trans* = 9-*cis* βC and 13-*cis* βC groups compared with the all-*trans* βC group; significance at $P < 0.05$, NS, not significant, $P > 0.05$.

TABLE 3

Vitamin A levels in liver, serum, lungs, adrenals, spleen, and kidneys from gerbils 6 h after oral doses of all-trans, 9-cis, or 13-cis β-carotene (βC) in cottonseed oil[1]

	Serum	Liver	Lung	Adrenal	Spleen	Kidney
	μmol/L	*nmol*	*nmol/g*			
Treatment[2]						
Control	1.26	90 ± 63	138	11	14	2.4
All-*trans*-βC	1.42 ± 0.5	225 ± 57	198 ± 33	13 ± 1.0	12 ± 2.1	3.3 ± 0.1
9-*cis*-βC	1.38 ± 0.2	191 ± 50	186 ± 43	16 ± 2.5	11 ± 1.7	2.8 ± 0.3
13-*cis*-βC	1.44 ± 0.1	211 ± 45	150 ± 3	13 ± 0.1	11 ± 1.1	3.4 ± 0.3
Statistics[3]						
βC vs. control	NS	NS	NS	NS	NS	0.0042
cis vs. *trans*	NS	NS	NS	NS	NS	NS
13 *cis* vs. 9 *cis*	NS	NS	NS	NS	NS	0.0132

[1] VA was quantified using HPLC. For tissues in all of the βC groups and the control group for liver, values represent means ± SEM; for serum, $n = 5$ for the all-*trans* βC group and $n = 3$ for the 9-*cis* βC and 13-*cis* βC groups; for liver, $n = 5$ for the control group and $n = 7$ for the βC groups; for lung, adrenal, spleen and kidney, $n = 3$ (pools of tissues from 2 gerbils). Values for control groups for serum, lung, adrenal, spleen and kidney represent means of $n = 2$ (pools of tissues from 2 gerbils).

[2] Oil dose = 185 ± 2 mg (mean ± SEM); Control = cottonseed oil alone, all-*trans* βC oil contained 384 ± 3 nmol total βC/dose, 9-*cis* βC oil contained 391 ± 2 nmol total βC/dose, and 13-*cis* βC oil contained 386 ± 2 nmol total βC/dose.

[3] ANOVA with orthogonal coding; βC vs. Control = all βC groups compared with the Control group; *cis* vs. *trans* = 9-*cis* βC and 13-*cis* βC groups compared with the all-*trans* βC group; $P \leq 0.05$, NS, not significant, $P > 0.05$.

Although the reduction in the amount of *cis* βC in the small intestine of gerbils administered the *cis* βC oils may be due to isomerization of *cis* isomers of βC to the all *trans* isomer, it is possible that the reduction is also due to a preferential uptake of *cis* isomers of βC into the small intestine over the 6-h period after dosing.

Indeed, we observed a small, but significant, upward shift in the proportion of *cis* isomers between the small intestinal contents and the intestinal mucosal scrapings of gerbils given the at*β*C oil and the 9*c*βC oil. Boileau and co-workers (42) reported a similar enhancement of *cis* lycopene isomers in mucosal tissue of ferrets, suggesting a selective incorporation of *cis* lycopene into bile acid micelles. Although a higher proportion of *cis* βC in the mucosa could suggest selective incorporation of *cis* βC into bile acid micelles before uptake, we did not observe this increase in *cis* βC in the mucosa of gerbils administered the 13*c*βC oil. In fact, the levels of total βC in the intestinal mucosal scrapings from gerbils given the at*β*C oil were higher than those of gerbils administered the *cis* βC oils, suggesting a preferential uptake of the all-*trans* isomer.

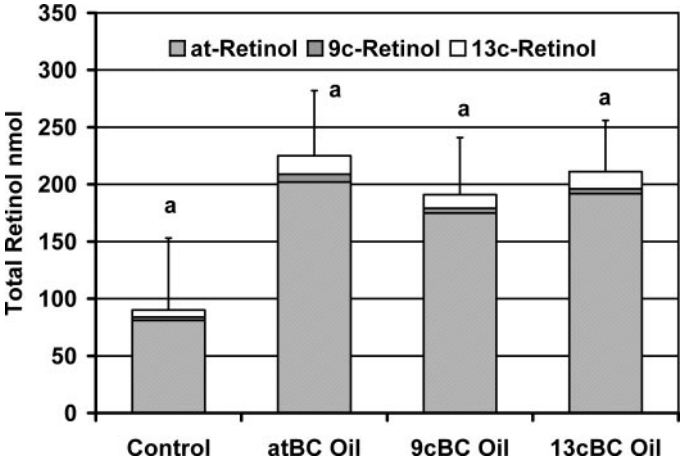

FIGURE 3 Vitamin A content in livers of gerbils orally administered no β-carotene (Control), all-*trans* β-carotene (at*β*C oil), 9-*cis* β-carotene (9*c*βC oil) or 13-*cis* β-carotene (13*c*βC oil). Gerbils were killed 6 h after the dose (all-*trans* βC group, 384 ± 3 nmol total βC; 9-*cis* group, 391 ± 2 nmol total βC; 13-*cis* group, 386 ± 2 nmol total βC) and total retinol was quantified from saponified liver tissue using HPLC. Retinol isomers [all-*trans* (a*t*), 9-*cis* (9*c*) and 13-*cis* (13*c*)] were calculated as a percentage of total retinol. Values are means ± SEM, $n = 5$ for the control group and $n = 7$ for the βC groups. Means did not differ.

This observation is consistent with a report by Stahl and co-workers (22) in which a 10- to 50-fold higher accumulation of at*β*C in chylomicrons in humans was observed after ingestion of a 1:5 mixture of at*β*C and 9*c*βC. This group suggested that an isomer-selective mechanism may be operative in the intestinal mucosal cell, involving specific uptake or incorporation steps, which excludes the 9-*cis* isomer from accumulation into chylomicrons (22). Of course, it is also possible that *cis* isomers are preferentially absorbed, perhaps 13-*cis* more than 9-*cis*, and then more rapidly taken up by tissues compared with the all-*trans* isomer. Johnson and co-workers (9) reported that at*β*C reached a higher postprandial concentration in serum, but that 9*c*βC reached peak levels sooner, after a single dose of either at*β*C or a mixture of all-*trans* and 9-*cis* βC in men. Their hypothesis was that these findings were due to either poor absorption or very rapid tissue uptake of 9*c*βC.

In addition to differences in intestinal uptake, there is evidence to support differential conversion of βC isomers to their respective VA metabolites in the intestinal mucosa, resulting in different levels of βC isomers and their metabolites at a given time point. Using in vitro enzyme preparations of rat intestine, Nagao and Olson (18) reported that at*β*C was the preferred substrate for cleavage to retinal, the direct product of this reaction, compared with 9*c*βC and 13*c*βC. In addition, 9*c*βC was converted to a mixture of 9-*cis*, all-*trans* and 13-*cis* retinals, whereas at*β*C and 13*c*βC were converted to all-*trans* retinal. Moreover, the rates of cleavage of 9*c*βC and 13*c*βC relative to at*β*C, i.e., 6.8 and 1.8%, respectively, in rat small intestine reported by this group suggest that only small amounts of *cis* isomers of βC are actually cleaved to

retinal (18) and thus may be poor precursors of VA compared with atβC.

In humans, elevated serum levels of atβC and 13cβC have been reported in response to intake of atβC (5–9). We found similar elevated levels of atβC and 13cβC in serum and liver from gerbils administered the atβC oil. The proportions of *cis* βC in serum (13.4%) and liver (14.1%) were not different; although they are higher, they are most consistent with that in the atβC oil dose (2.2%) compared with other fractions. The predominant βC isomer in livers from the atβC group was the all-*trans*, which was 86% of the total βC. In rats, 9cβC contributed up to 10% of the total βC in liver after a dose of pure atβC dissolved in soybean oil (22). The accumulation of 9cβC in liver has also been reported in chicks and rats after feeding synthetic βC (13).

The observation of low serum levels and relatively high tissue levels of 9cβC in gerbils given the 9cβC oil in our study are also consistent with observations in tissues of humans and animals (14,15,17). In fact, the total βC in serum from gerbils given either of the *cis* βC oils was significantly lower than that of those administered the atβC oil, and the predominant βC isomer in serum from gerbils gavaged with the *cis* βC oils was all-*trans* (57% in 13cβC oil, 83% in 9cβC oil). Total βC in livers of gerbils given the 9cβC and 13cβC oils were significantly higher than that of gerbils given the control oil. 9-*cis* βC was the predominant isomer in livers (79% of total liver βC) from the 9cβC group. In contrast, the all-*trans* isomer was 63% of the total βC in livers from 13cβC group, and the 9-*cis* isomer was 21%. This value is similar to that reported in human liver samples from autopsy patients, in which 9cβC was up to 25% of the total βC (12).

The use of a 6-h time point in our study resulted in measurable tissue accumulations of βC isomers in all βC groups. We estimated the bioavailability of the dose of βC isomer absorbed and stored in the liver from each group on the basis of the gain in liver βC and VA. The relative bioavailability was estimated to be 38% for the atβC oil group, 27% for the 9cβC oil group and 32% for the 13cβC oil group. Although these values assume that liver βC is an available source of VA, the actual contribution of accumulated liver βC to the VA needs of the body is unclear (31).

We observed a trend for greater liver VA storage in all βC isomer-treated groups compared with the control group 6 h after the dose was given ($P = 0.63$; Fig. 3). It is particularly noteworthy that the isomeric composition of liver VA was consistent in gerbils given either of the βC isomer oils, ranging from 87 to 90% all-*trans*, 6–7% 13-*cis*, and 2–5% 9-*cis*. Levin and Mokady (43) observed a similar isomeric composition in livers from chicks fed diets containing a synthetic mixture of atβC and 9cβC for 1 wk. Retinol stereoisomers have also been reported in tissues of rats (44) and fish (45).

The predominance of the all-*trans* isomer of VA in the liver may be due to the fact that all-*trans* βC is the preferred substrate for cleavage. Alternatively, isomerization may occur during cleavage. It is also possible that all-*trans* retinol is the preferred substrate for the lecithin retinyl acyl transferase enzyme, which esterifies retinol to fatty acids before storage. Straight-chain all-*trans* retinyl esters may provide greater storage efficiency than bent-shaped *cis* retinyl esters. Based on the relative VA biopotency of retinol isomers (100% for all-*trans*, 76% for 13-*cis*, 19% for 9-*cis*) estimated by Weiser and Somorjai (46) using rat vaginal smear assays, the isomeric composition of liver VA should also be considered when evaluating the VA biopotency of βC isomers.

Although we did not evaluate the isomeric composition of VA in serum, You and co-workers (24) reported substantial levels of [^{13}*c*]all-*trans* retinol in human serum after a dose of [^{13}C]9cβC, suggesting that low serum levels of 9cβC may be because of 9cβC isomerization occurring in vivo at some undetermined point during the digestive, uptake or absorptive processes. Interestingly, this group further hypothesized that *cis*-to-*trans* isomerization of 9cβC before its secretion in the bloodstream may be a mechanism to limit the supply of a 9-*cis* retinoid precursor to tissues, while not totally sacrificing the VA value of this isomer.

In summary, because of its apparent preferential uptake, transport and tissue accumulation, atβC appears to be a more bioavailable βC isomer than the 9cβC and 13cβC isomers 6 h after a dose in gerbils. Although the apparent bioavailability of βC isomers was differentiated in tissues within 6 h, liver VA stores were not different among the groups. Yet, the mean total liver VA followed an increasing numerical trend for the groups: atβC > 13cβC > 9cβC. Although we can not assign a relative VA biopotency for atβC, 9cβC and 13cβC from these data, we evaluated their biopotencies in a subsequent study using a series of doses over a 7-d period (47).

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