The Relative Vitamin A Value of 9-*cis* β-Carotene Is Less and That of 13-*cis* β-Carotene May Be Greater than the Accepted 50% That of All-*trans* β-Carotene in Gerbils[1]

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ABSTRACT The effectiveness of β-carotene (βC) as a vitamin A (VA) precursor may be influenced by the proportions of *cis* isomers of βC consumed in the diet. Although the metabolic fates of *cis* isomers of βC are poorly understood, their retinol equivalency has been assigned a value 50% that of all-*trans* (a*t*) βC. A dose-response design was used to estimate the relative VA value (VAV) of a*t*βC, 9-*cis* (9*c*) βC and 13-*cis* (13*c*) βC in gerbils using total liver retinol as a measure of VAV. Ten groups of gerbils received a daily dose of oil with or without βC isomer by gavage for 7 d. Nine groups (n = 5) were divided equally among the three βC dosing treatments with each isomer provided at 141, 275 and 418 nmol/d. Total liver VA (171–259 nmol) in gerbils administered a*t*βC was higher than total liver βC (25–53 nmol). Stores of VA and βC in livers from gerbils administered a*t*βC were higher than stores of VA and βC in livers from those given 9*c*βC or 13*c*βC. The relative VAV of *cis* βC isomers was estimated by comparing slopes of dose-response lines of all three βC isomers using a*t*βC as a reference. Total liver VA and βC increased linearly ($P < 0.05$) with increasing βC intake in gerbils gavaged with all three βC isomer oils. The relative VAV of 9*c*βC was less (38%) and 13*c*βC was more (62%) than the assigned value of 50% that of a*t*βC. Thus, the proportions of *cis* isomers of βC contained in a food could negatively affect the vitamin A value of the diet. J. Nutr. 132: 2709–2712, 2002.

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

In recent years, the efficacy of plant-based diets to provide vitamin A (VA)[3] has been duly questioned as a result of reported poor absorption of β-carotene (βC) from vegetables consumed by marginally VA-deficient populations (1). The actual contribution of βC to the VA requirement may be influenced in part by the proportions of *cis* isomers of βC in the diet. Although all-*trans* βC (a*t*βC) is accompanied by small amounts of 9-*cis* βC (9*c*βC) and 13-*cis* βC (13*c*βC) in raw fruits and vegetables (2), the proportions of *cis* isomers in these foods may be greatly increased during processing (2,3).

The dietary intake of *cis* isomers of βC may be substantial, but their metabolic fate is poorly understood. C*is* isomers of βC have been assigned a retinol equivalency that is 50% that of a*t*βC (4). Yet, the efficiencies of 9*c*βC and 13*c*βC as VA precursors in rats were >50% using a storage assay of liver VA (5) and <50% using a growth assay (6). Although we previously observed dramatic differences in the quantities of βC in tissues of gerbils 6 h after a single dose of a*t*βC, 9*c*βC and 13*c*βC, this length of time did not allow for estimation of the relative VA value (VAV) of each isomer (7). Thus, the objective of this study was to determine the relative VAV of a*t*βC, 9*c*βC and 13*c*βC. Total liver VA was quantified from groups of gerbils orally administered doses of oil containing increasing quantities of each βC isomer for 7 d. VAV was then estimated by comparing slopes of dose-response lines using a*t*βC as the reference.

MATERIALS AND METHODS

A detailed description of the materials and methods was reported previously (7). Male Mongolian gerbils (*Meriones unguiculatus*, n = 57, age 28 d, 23 ± 2 g body weight, Charles River Laboratories, Raleigh, NC) were given free access to water and a modified AIN-93G diet formulated without VA or βC (7) for 56 d. All procedures were approved by the University of Illinois Laboratory Animal Care Advisory Committee. Ten gerbils were killed after depletion to establish baseline tissue VA.

The remaining gerbils were assigned to one of 10 groups and repleted for 7 d by gastric intubation with a daily, oral dose of crystalline βC solubilized in cottonseed oil or cottonseed oil alone. Nine groups (n = five) were divided equally among three βC dosing treatments; a*t*βC, 9*c*βC or 13*c*βC was provided at 141, 275 and 418 nmol/d. A control group (n = 3) received oil without βC. To ensure solubility of the βC crystals in the oil, 10 oils (3 oils/βC isomer, plus a control oil) were freshly prepared every 2 d during the treatment period. Total βC intake for each gerbil was the 7-d intake of βC isomer from each oil. The gerbils were killed 24 h after the last dose, followed by collection of blood and organ tissues. Total VA and βC were quantified by HPLC in serum, liver and lung tissue from each individual gerbil. Concentrations of VA and βC were quantified in adrenal glands, spleen and kidneys from two pooled groups of tissue (2 gerbils/pooled group).

Data were analyzed using the Statistical Analysis System (version 6.12, SAS Institute, Cary, NC). Data for each βC isomer oil were fitted to linear and quadratic models evaluating total liver retinol as a function of total βC intake. If the quadratic model was significant, the data points for the highest dose were omitted, and the analysis was repeated. If the quadratic model was not significant, all data

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[3] Abbreviations used: a*t*βC, all-*trans* β-carotene; βC, β-carotene; 9*c*βC, 9-*cis* β-carotene; 13*c*βC, 13-*cis* β-Carotene; RAE, retinol activity equivalent; VA, vitamin A; VAV, vitamin A value.

points were used to fit the data to a regression line. The ratio of slopes of the lines, using atβC as a reference, provided estimates of the relative VAV of βC *cis* isomers expressed as a percentage. All values presented in the text are means ± SEM.

RESULTS

The purities of the atβC, 9*c*βC and 13*c*βC oils were 96 ± 1%, 99 ± 0.4% and 91 ± 2%, respectively. The total βC per dose of oil (185 ± 2 mg) was 141 ± 2, 275 ± 3 and 418 ± 4 nmol, respectively. The growth rate of gerbils during the depletion period was not different from that reported previously (7).

The baseline tissue VA levels were as follows: *1*) liver, 36 ± 4 nmol (12 ± 2 nmol/g); *2*) serum, 0.6 ± 0.1 μmol/L; *3*) lung, 25 ± 3 nmol; *4*) adrenal, 22 ± 2 nmol/g; *5*) spleen, 18 ± 2 nmol/g; 6) kidney, 4 ± 1 nmol/g. Although the liver VA status suggests a high risk for VA deficiency, the gerbils did not exhibit ill health or physical signs of VA deficiency during the 56-d depletion period. Liver βC content was 0.9 ± 0.7 nmol (0.3 ± 0.2 nmol/g). βC was not detected in other tissues.

Total liver VA content increased linearly ($P < 0.05$) in gerbils administered atβC- (Fig. 1A), 9*c*βC- (Fig. 1B) and 13*c*βC- (Fig. 1C) containing oils. The quadratic ($P = 0.006$) response to the data was significant for the atβC oil, but not for the *cis* βC oils. Thus, the data points for the highest doses of atβC were omitted, thereby isolating the linear phase of the response. All data points were used to fit the linear regression line for the *cis* βC oils. The equations for the lines were: atβC oil: $y = 0.089x + 63.5$ ($r^2 = 0.80$); 9*c*βC oil: $y = 0.034x + 44.8$ ($r^2 = 0.65$); and 13*c*βC oil: $y = 0.055x + 48.0$ ($r^2 = 0.81$). A comparison of the slopes of these lines resulted in a relative VAV of 38% for 9*c*βC and 62% for 13βC, compared with atβC.

Lung VA was 1–2 times greater than that of controls (**Table 1**). The isomeric profiles of retinol in the livers of gerbils after the 7-d dosing period of atβC, 9*c*βC and 13*c*βC were similar (i.e., 88–93% at, 5–9% 13c, and 2–3% 9c).

βC was detected only in liver, adrenal and spleen of gerbils administered βC isomers (**Table 2**). Although liver VA was higher than liver βC, we observed a similar linear increase ($P < 0.05$) in liver βC in gerbils given βC isomers. Relative to gerbils given atβC (i.e., 100%), total liver βC was 67% in gerbils administered 9*c*βC and 42% in those given 13*c*βC. The major βC isomer in tissues was atβC in gerbils administered atβC or 13*c*βC, but the principal isomer in gerbils gavaged with 9*c*βC was 9*c*βC.

DISCUSSION

We estimated the relative effectiveness of atβC, 9*c*βC and 13*c*βC to enhance liver VA storage in gerbils given daily oral doses of oil containing increasing quantities of each βC isomer by gastric intubation for 7 d. Relative VA value (VAV) was estimated by comparing slopes of linear regression lines evaluating total liver VA as a function of total βC intake. The relative VAV of 9*c*βC and 13*c*βC was estimated to be 38 and 62%, respectively, that of atβC.

Previous work investigating the VA biopotencies of *cis* isomers of βC used either a growth or a liver VA storage assay (6,8,9). Deuel and co-workers (6) fed small graded doses of isomers of βC for 28 d to rats using a growth assay. Guggenheim and Koch (8) found that the liver storage assay was less time consuming and provided more precision than the growth assay, but required the administration of much larger doses of βC. In our study, the use of gerbils provided the advantages of both assays in that we achieved increasing storage of liver VA using physiologic doses of βC isomers according to Pollack and co-workers (10) given over 7 d.

The depletion of tissue VA before carotene dosing has also been used in evaluating the VA biopotency of carotene iso-

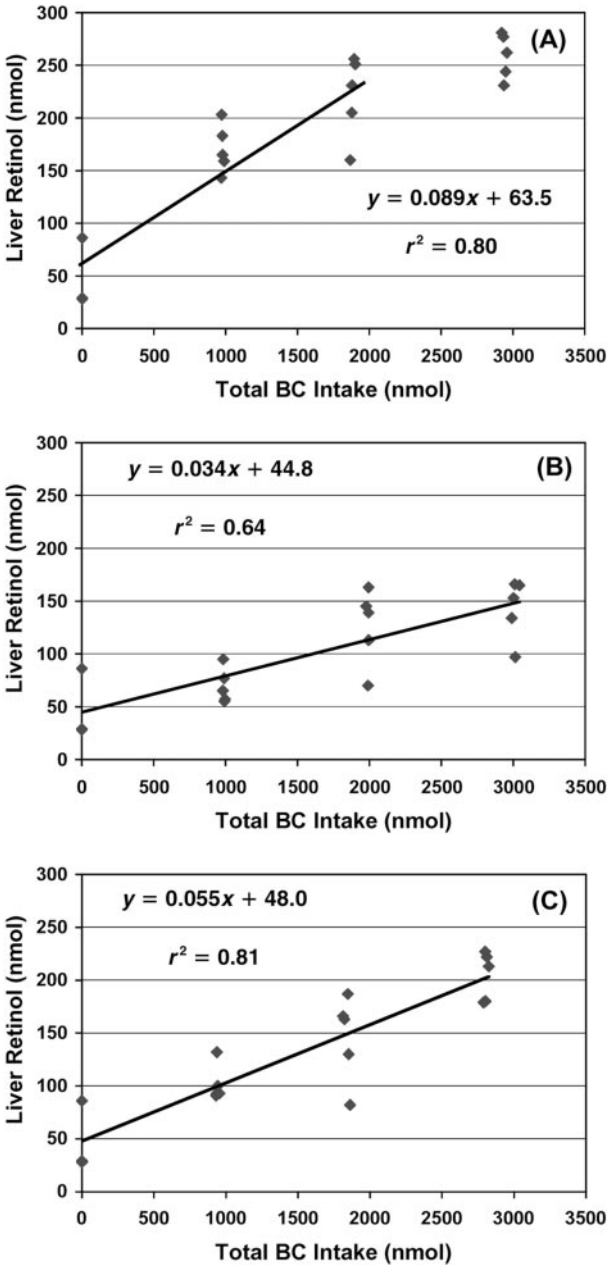

FIGURE 1 Dose responses of liver vitamin A (VA) content in gerbils gavaged with cottonseed oil containing increasing levels of (*A*) all-*trans* β-carotene (atβC), (*B*) 9-*cis* (9c) βC or (*C*) 13-*cis* (13c) βC. The total βC/dose (nmol ± SEM) of oil (185 ± 2 mg) for each dose level was 141 ± 2, 275 ± 3 and 418 ± 4 nmol, respectively. Gerbils received a daily dose of oil by gastric intubation for 7 d and were killed 24 h after the last dose. Total liver VA was quantified from saponified tissue using HPLC. Total βC intake for each gerbil was the 7-d intake from each βC isomer oil. Equations were derived using linear regression analysis. Total liver VA contents from individual gerbils in the control group were 28, 29 (overlapping values) and 86 nmol. A quadratic fit to the data was significant ($P = 0.006$) for the atβC oil (A). Thus, the data points for the highest dose level were omitted and the line was fitted using the remaining data points.

TABLE 1

Vitamin A levels in serum, liver, lungs, adrenal, spleen and kidneys from gerbils orally administered cottonseed oil alone (control) or with three increasing levels of all-trans β-carotene (βC), 9-cis (9c)βC or 13-cis (13c)βC solubilized in cottonseed oil[1]

βC Group	βC dose[2]	Serum	Liver[3]	Lung	Adrenal[4]	Spleen[4]	Kidney[4]
	nmol/d	*μmol/L*	*nmol*		*nmol/g*		
Control	0	0.58 ± 0.08	48 ± 6	34 ± 5	22	22	4
All-*trans*	141	1.02	171	76	24	17	7
All-*trans*	275	1.12	221	82	21	21	6
All-*trans*	418	1.17	259	90	22	19	8
9-*Cis*	141	1.16	70	41	20	21	6
9-*Cis*	275	1.13	126	72	24	19	8
9-*Cis*	418	1.21	143	61	22	20	6
13-*Cis*	141	1.22	102	63	22	17	6
13-*Cis*	275	1.39	146	66	25	14	6
13-*Cis*	418	1.06	204	83	19	21	6
Pooled SEM	9	0.10	13	8	—	—	—

[1] Values are means ± SEM (control, $n = 3$), means with pooled SEM βC-groups ($n = 5$) or means alone when $n = 2$ (adrenal, spleen, kidney).
[2] The oil dose (nmol ± SEM) = 184 ± 2 mg. The *at*βC oil, 9*c*βC oil and 13*c*βC oil contained 96.0 ± 2% *at*βC, 99.0 ± 1% 9*c*βC and 91.0 ± 3% 13*c*βC, respectively.
[3] Linear response to βC isomer dose ($P < 0.05$).
[4] Values are means of 2 pools of tissues from 2 gerbils for βC groups and the value in one pool of tissue from two gerbils for the control group.

mers. Johnson and Baumann (9) depleted rats (24–30 d) until they failed to gain weight over a 7-d period before repletion with carotenes. These rats exhibited the classic eye symptoms of VA deficiency. Because the liver may be substantially depleted of VA before any weight loss occurs, it has been suggested that carotene should be given before VA depletion results in a weight plateau (5). During the depletion period in our study, gerbils were still gaining weight before βC repletion.

Our VAV estimates of 38% for 9*c*βC and 62% for 13*c*βC suggest that 9*c*βC may have less and 13*c*βC more than the previously assumed retinol equivalency of 50% for *cis* isomers of βC. Our data strongly suggest that both *cis* isomers have lower VA biopotencies than a*t*βC, and also that 9*c*βC has a lower VAV than a*t*βC, a lower VAV than 13*c*βC, and it has a VAV lower (relative to a*t*βC) than 50%. The results are less persuasive that 13*c*βC has a VAV >50% of a*t*βC. Indeed, calculation of slope values ($\Delta y/\Delta x$) for responses between the control (no βC dosing) and the first increment of βC isomers yielded slopes (nmol total liver VA/nmol total βC intake) of 0.872, 0.156 and 0.383 for a*t*βC, 9*c*βC and 13*c*βC, respectively. Translating these slopes to VA biopotencies gives VAV (relative to a*t*βC) of only 18% for 9*c*βC and 44% for 13*c*βC. Thus we conclude that the relative biopotency of 9*c*βC is both lower than that of 13*c*βC and <50% that of a*t*βC, whereas the relative biopotency of 13*c*βC is ≥50% that of a*t*βC.

It is clear from rat studies and this gerbil study that the VA biopotencies are lower for *cis* isomers of βC than a*t*βC and that 9*c*βC is consistently ranked lower than 13*c*βC. However, the magnitude of difference from the assigned retinol equivalency of 50% that of a*t*βC for these βC *cis* isomers varies among studies. In rats, Deuel and co-workers (6) and Johnson and Baumann (9) reported a VAV notably <50% for 9*c*βC (i.e., 33 and 38%, respectively), and, in both studies, the VAV for 13*c*βC was essentially ~50% that of a*t*βC (i.e., 48 and 53%, respectively). Estimates of the VAV of 9*c*βC reported by Weiser and co-workers (11) were also consistently <50% that of a*t*βC regardless of whether they used ovariectomized females in a growth assay (23%), a vaginal epithelial protection assay (26%) or VA-deficient males in a growth assay (23%).

In contrast to these studies, Sweeney and Marsh (5) reported a VA biopotency much >50% of a*t*βC for both 9*c*βC (61%) and 13*c*βC (74%) in rats (5). They also observed a wide variation in liver VA in rats of similar weight fed the same βC isomer at the same level. We observed similar variation in liver VA in gerbils, but lower relative VA biopotencies for 9*c*βC and 13*c*βC than did Sweeney and Marsh. Yet, our gerbil study is the only one reporting relative VA biopotencies of 9*c*βC and 13*c*βC, which appear to be notably lower and higher, respectively, than 50% that of a*t*βC. In addition,

TABLE 2

β-Carotene (βC) levels in liver, adrenal and spleen from gerbils orally administered cottonseed oil alone (control) or with three increasing levels of all-trans (at), 9-cis (9c) or 13-cis (13c) βC[1,2]

βC group	βC dose[3]	Liver[4]	Adrenal	Spleen
	nmol/d	*nmol*	*nmol/g*	
Control	ND[5]	ND	ND	ND
All-*trans*	141	25	1.7	1.9
All-*trans*	275	45	4.5	2.7
All-*trans*	418	53	4.1	4.6
9-*Cis*	141	22	2.7	1.8
9-*Cis*	275	33	4.9	2.1
9-*Cis*	418	51	5.5	2.5
13-*Cis*	141	8	0.7	0.7
13-*Cis*	275	16	1.0	0.9
13-*Cis*	418	28	0.7	1.7
Pooled SEM	9	4	—	—

[1] Values are means ± SEM (control, $n = 3$), means with pooled SEM (βC groups, $n = 5$) or means alone (adrenal, spleen, kidney; $n = 2$).
[2] Values are means of 2 pools of tissue from 2 gerbils for βC groups and the value in 1 pool of tissue from 2 gerbils for the control group.
[3] The oil dose (nmol ± SEM) = 184 ± 2 mg. The total βC per dose (nmol ± SEM) was: 141 ± 2, 245 ± 3 and 418 ± 4. The all-*trans* (*at*)βC oil 9-*cis* (9*c*)βC oil and 13-*cis* (13*c*)βC oil contained 96.0 ± 2% *at*βC, 99.0 ± 1% 9*c*βC and 91.0 ± 3% 13*c*βC, respectively.
[4] Linear response to βC isomer dose ($P < 0.05$).
[5] ND, not detected.

the difference between the relative VA biopotencies of 9*c*βC and 13*c*βC is greater in gerbils than that reported in rats, which may be a result of differences in βC metabolism between rats and gerbils. Differences in βC metabolism are common among animals and between humans and animals. Thus, the applicability of our data in gerbils, as with other animal models, may be limited when comparing it with humans.

The lower VA biopotencies of 9*c*βC and 13*c*βC relative to a*t*βC could be because of destruction in the digestive tract before absorption or slower absorption leading to greater loss in the feces (12,13). Yet, high recoveries of *trans* and *cis* βC have been reported in feces of rats regardless of the isomer fed (5,12), suggesting little destruction but a high extent of isomerization of βC in the digestive tract. Previously, we observed preferential absorption, transport and accumulation of a*t*βC in tissues of gerbils 6 h after a dose of a*t*βC vs. 9*c*βC and 13*c*βC (7). Similar preferential accumulation of a*t*βC has been reported in human serum (14) and chylomicrons (15). It is possible that the VA biopotency attributed to the *cis* isomers of βC depends on the extent to which these isomers are converted to a*t*βC before or after absorption, and for the most part, only that portion isomerized to a*t*βC is cleaved to form VA. We have suggested that a*t*βC appears to be the preferred substrate for βC cleavage in gerbils (7).

A few studies support in vivo isomerization of *cis* isomers of βC. You and co-workers (13) reported that ^{13}C-labeled 9*c*βC yielded an equivalent amount of ^{13}C-labeled all-*trans* retinol in human serum compared with the same oral dose of ^{13}C-labeled a*t*βC, suggesting that isomerization occurred during digestion, uptake and/or absorption. Kemmerer and Fraps (12) reported progressive, nonspecific isomerization of 9*c*βC in the digestive tracts of rats after a dose of 9*c*βC. We also reported nonspecific isomerization of βC isomers in the digestive tracts of gerbils (7). A 50:50 *cis*:*trans* ratio of βC was observed in the contents of the small intestine in gerbils administered 13*c*βC compared with a 70:30 *cis*:*trans* ratio of βC in gerbils given 9*c*βC and a 20:80 *cis*:*trans* ratio in gerbils given a*t*βC. Thus, the proportion of the all-*trans* isomer of βC in the small intestinal contents of gerbils gavaged with 13*c*βC and 9*c*βC was 63 and 38% that contained in the small intestinal contents of gerbils administered a*t*βC (7). The presence of more a*t*βC in the small intestine of gerbils given 13*c*βC compared with 9*c*βC (7), combined with the results from the current study showing a higher relative VAV for 13*c*βC than 9*c*βC, suggests that isomerization before cleavage plays a major role in the VA biopotency of 13*c*βC and 9*c*βC.

The demonstration that the relative VAV of 9*c*βC was substantially less and that of 13*c*βC was somewhat more than the assigned value of 50% that of a*t*βC may justify a revised retinol activity equivalent (RAE) for these isomers. On the basis of the current RAE (4), 1 μg retinol = 12 μg of a*t*βC from foods. Rather than the currently assigned RAE of 24 μg for provitamin carotenoids other than a*t*βC, the RAE of 13*c*βC and 9*c*βC can be calculated to be ~19 and ~32 μg, respectively, on the basis of the relative VAV of these isomers (62 and 38%, respectively).

When vegetables are thermally processed, 30–50% of a*t*βC may be converted to *cis* isomers (2,3). The proportions of a*t*βC, 13*c*βC and 9*c*βC in canned carrots were reported to be 73, 19 and 8%, whereas those of canned spinach were 58, 15 and 25%, respectively (2). On the basis of these proportions of isomers and the revised RAE values for 13*c*βC and 9*c*βC, the actual VA activity of canned carrots (with higher proportions of 13*c*βC than 9*c*βC) is higher and that of canned spinach (with higher proportions of 9*c*βC than 13*c*βC) is lower than expected using the current RAE. Although there are many factors that affect βC bioavailability, the actual contribution of βC to the VA requirement may be strongly influenced by the proportions of *cis* isomers of βC consumed in the diet. Thus, isomers of βC, especially from cooked foods, have to be considered in evaluating the VA biopotencies of βC-rich vegetables consumed by marginally VA-deficient populations around the world.

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