Relative vitamin A values of 9-*cis*- and 13-*cis*-β-carotene do not differ when fed at physiological levels during vitamin A depletion in Mongolian gerbils (*Meriones unguiculatus*)

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Abstract

Provitamin A biofortification of staple crops may decrease the prevalence of vitamin A (VA) deficiency if widely adopted in target countries. To assess the impact of processing methods on the VA value of plant foods, the unique bioefficacies of *cis*-βC isomers (formed during cooking) compared with all-*trans* (a*t*) β-carotene (βC) must be determined. The bioefficacies of 9-*cis* (9*c*)- and 13-*cis* (13*c*)-βC isomers were compared with those of the a*t*-βC isomer and VA positive (VA+) and negative (VA−) controls in VA-depleted Mongolian gerbils (*Meriones unguiculatus*) in two experimental studies (study 1, *n* 56; study 2, *n* 57). A 3- or 4-week depletion period was followed by a 3- or 4-week treatment period in which the groups received oral doses of the 9*c*-, 13*c*- or a*t*-βC isomers in cottonseed oil (study 1, 15 nmol/d; study 2, 30 nmol/d). In study 1, the βC isomers did not maintain baseline liver VA stores in all groups (0·69 (sd 0·20) μmol/liver) except in the VA+ group (0·56 (sd 0·10) μmol/liver) (*P*=0·0026). The βC groups were similar to the VA+ group, but the 9*c*- and 13*c*-βC groups did not differ from the VA− group (0·39 (sd 0·09) μmol/liver). In study 2, the βC isomers maintained baseline liver VA stores in all the βC groups (0·35 (sd 0·13) μmol/liver), and in the VA+ group, the VA supplement (0·54 (sd 0·19) μmol/liver) exceeded the baseline VA status (0·38 (sd 0·15) μmol/liver) (*P*<0·0001); however, the 9*c*-βC group did not differ from the VA− group (0·20 (sd 0·07) μmol/liver). *In vivo* isomerisation of βC was confirmed in both experimental studies. Lower VA bioconversion factor values were obtained for the *cis*-βC isomers in study 2 when compared with study 1, but higher values were obtained for the a*t*-βC isomer. Dose and VA status clearly affect bioconversion factors. In conclusion, the *cis*-βC isomers yielded similar liver VA stores to the a*t*-βC isomer in Mongolian gerbils, and liver VA stores of the 9*c*- and 13*c*-βC groups did not differ when the doses were provided at physiological levels over time in two studies.

Key words: β-Carotene: Bioavailability: Bioconversion: Isomer effects

β-Carotene (βC) is one of the most common carotenoids in the human diet[1], and its primary function in the body is provitamin A activity[2]. All-*trans* (a*t*)-βC is the most abundant isomer in fresh plant foods, but thermal processing can increase the proportion of 9-*cis* (9*c*)- and 13-*cis* (13*c*)-βC isomers up to 50%[3]. Little is known about the vitamin A (VA) efficacies of the *cis*-βC isomers, which have been collectively assigned a bioavailability equivalent to half that of a*t*-βC[4]. Therefore, it is imperative to determine the unique bioefficacies of the *cis*-βC isomers to better understand the impact of thermal processing methods on the VA value of plant foods.

VA deficiency is a global health concern, affecting 190 million children under 5 years of age[5]. The introduction of provitamin A carotenoid-biofortified staple crops is a novel approach to alleviate VA deficiency and prevent issues observed with preformed VA supplementation and fortification, such as cyclical liver stores and hypervitaminosis risk[6]. However, staple crops undergo thermal processing before consumption; thus, the impact of isomerisation may need to be considered when establishing biofortification targets in order to ensure adequate delivery of VA to target populations.

A study by Deming *et al.*[7] has determined the individual bioavailabilities of 9*c*- and 13*c*-βC isomers in gerbils to be 38 and 62% that of a*t*-βC, respectively, using doses that ranged between 141 and 418 nmol βC/d or 132 and 392 nmol VA/d according to the Institute of Medicine (IOM)'s recommended values of 2 μg a*t*-βC to 1 μg retinol activity equivalents for supplemental βC[4]. These orally administered doses greatly exceeded the utilisation rate (10·8 nmol VA/100 g body weight/d[8]) and the current recommendation of the

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National Research Council for a typical adult gerbil (15·1 nmol VA/d, assuming an average feed intake of approximately 6 g[9]), and therefore they are not reflective of typical dietary intake that would be achieved from staple crops. Howe et al.[10] found that βC-enhanced cassava feeds providing 10·9–27·5 nmol βC/d maintained VA status in gerbils despite 40–50 % cis-βC isomer content in cassava. Additionally, in an in vitro study using isomeric mixtures, the 9c-βC isomer enhanced overall total carotene in solution and resulted in constant or enhanced micellar incorporation[11]. Thus, the objective of the present study was to compare the bioefficacies of the 9c-, 13c- and at-βC isomers in VA-depleted gerbils using doses reflective of dietary intakes from staple foods. Conversion to retinol was determined using total liver VA stores compared with a positive preformed retinol-supplemented group and a negative VA-depleted group.

Materials and methods

Chemicals and dose preparation

Crystalline at-βC (Sigma-Aldrich), 9c-βC and 13c-βC (gifts from Hoffmann-La Roche, Inc.) were prepared for purification by dissolution in n-hexane. βC isomers were purified on an open column using Al as the stationary phase and n-hexane as the mobile phase. The purities of all βC isomers were confirmed as >99 % with HPLC. For stock solutions, purified βC in hexane was condensed to 10 ml under N_2, mixed with 20 ml cottonseed oil in an amber vial, and dried to remove remaining hexane and ensure clear solutions. The removal of hexane was confirmed by repeated weighing until a constant weight was achieved. Using spectrophotometry, concentrations were calculated with $E_{1cm}^{1\%} = 2590$ for the at-βC isomer at 450 nm, $E_{1cm}^{1\%} = 2550$ for the 9c-βC isomer at 445 nm and $E_{1cm}^{1\%} = 2090$ for the 13c-βC isomer at 443 nm[12]. Specific volumes of stock solution were pipetted into amber vials containing the appropriate amount of oil to achieve the target dose and were mixed by sonication; final doses were validated by dissolution of the dose in hexane followed by spectrophotometry where the reference cell contained an equal amount of oil dissolved in hexane. Doses were divided and stored at −70°C to minimise isomerisation. Lack of isomerisation during dose preparation was confirmed by running an aliquot of the oil dose dissolved in isopropanol on the HPLC system. A new bottle was opened at each study week.

Study design

Male Mongolian gerbils (Meriones unguiculatus) (study 1, n 56; study 2, n 57; Charles River Laboratories), aged 34 d old, were group-housed (n 2–3 per cage) throughout the study. Room temperature and humidity were held constant (12 h light–dark cycle). Gerbils were acclimatised to the dosing procedure before the start of the treatments by orally administering 40 μl cottonseed oil/d using a positive displacement pipette. Gerbils were weighed on a daily basis for about 2 weeks and thereafter were weighed twice per week. Animal handling procedures were approved by the College of

Agriculture and Life Sciences Animal Care and Use Committee at University of Wisconsin-Madison.

Gerbils consumed ad libitum water and VA- and carotenoid-free, semi-purified, powdered feed for the duration of the study. The feed was designed in consultation with a feed nutritionist (Harlan-Teklad) to meet the gerbils' energy and nutrient requirements without any known source of VA or βC (Table 1). Feed intake was determined by measuring the feed in a clean bowl and subtracting the remainder after a 24 h period. After depletion (study 1, 3 weeks; study 2, 4 weeks), a baseline group (study 1, n 6; study 2, n 7) was killed via exsanguination while under isoflurane anaesthesia to determine initial serum and liver VA concentrations. The remaining cages were sorted into weight-matched treatment groups (n 10 per group). To ensure 90 % power with a 5 % significance level, nine gerbils per treatment group were necessary to detect a 30 nmol difference in liver retinol concentration, assuming a standard deviation of 20 nmol. The sample size was increased to ten gerbils per group to account for the animals that may fail to thrive during the experimental period or to mitigate outliers.

In the present study, three groups received daily oral doses of at-, 9c- or 13c-βC in cottonseed oil, and the other groups received doses of VA-free cottonseed oil (VA−) or retinyl acetate in cottonseed oil (VA+). In study 1, a low βC dose of 15 nmol/d was designed to provide twice the molar equivalent of the daily VA utilisation rate of a 70 g gerbil[8]. In study 2, a moderate βC dose of 30 nmol/d was designed to provide twice the molar equivalent of the current VA recommendation of 2·5 nmol/g feed[9], assuming a typical feed intake of approximately 6 g/d. The retinyl acetate dose provided the proportion of 1 μg retinol:2 μg at-βC in accordance with the current equivalents of the IOM for oil βC supplements[4]. Following a 3-week (study 1) or a 4-week (study 2) treatment period, gerbils were killed. Blood was allowed to clot at room temperature for 30 min and centrifuged for 15 min at 2200 **g** at 4°C in vacutainer tubes (Becton Dickinson). Serum and liver samples were stored at −70°C.

Table 1. Composition of a vitamin A (VA)- and carotenoid-free gerbil feed

Ingredients	VA- and carotenoid-free feed (g/kg)
Casein, vitamin-free	200
L-Cys	3·0
Sucrose	360·5
Maltodextrin	120
Maize starch	150
Cottonseed oil	60
Cellulose	60
Mineral mix, AIN-93M-MX	35
Magnesium oxide	1·75
Calcium phosphate, dibasic	2·0
Vitamin mix*	5·0
Vitamin E acetate	0·242
Vitamin D_3	0·004
Choline bitartrate	2·5

* Provided per kg of purified diet: biotin, 0·4 mg; calcium pantothenate, 66·1 mg; folic acid, 2 mg; inositol, 110·1 mg; menadione, 49·6 mg; niacin, 99·1 mg; p-aminobenzoic acid, 110·1 mg; pyridoxine-HCl, 22 mg; riboflavin, 22 mg; thiamin-HCl, 22 mg; vitamin B_{12} (0·1 % in mannitol), 29·7 mg; ascorbic acid (97·5 %), 1016·6 mg.

Tissue extraction procedures

Samples were analysed under gold light to prevent photo-oxidation and isomerisation. C-23 *apo*-carotenol was used as the internal standard in all serum and liver samples. A modified procedure was used for the analysis of serum[13]. In study 1, 500 μl serum was analysed, reconstituted in 100 μl of methanol–dichloroethane (50:50), and 25 μl injected. In study 2, to assess the isomeric profile, 1000 μl serum was analysed, reconstituted in 80 μl of methanol–butanol (70:30), and 50 μl injected.

Liver (approximately 0·7 g) was ground with approximately 4 g sodium sulphate, extracted with dichloromethane, filtered and made up to 50 ml. For retinoid and carotenoid analyses, aliquots of 5 and 15 ml, respectively, were dried under N_2, reconstituted in 100 μl of methanol–dichloroethane (50:50), and 25 or 50 μl injected.

HPLC analyses

βC isomers were separated from the oil doses and liver samples using a C30 YMC carotenoid column (4·6 × 250 mm, 3 μm; Waters, Inc.) at 450 nm, as described previously[14], on a Waters HPLC system consisting of a 1525 binary HPLC pump, a 717 autosampler and a 996 photodiode array detector. The samples were held at 4°C in the autosampler to prevent isomerisation or degradation.

For serum analysis in study 1 and all liver analyses, retinol and retinyl esters were separated using a Resolve C18 column (3·9 × 300 mm, 5 μm; Waters, Inc.) at 325 nm. Solvent A was acetonitrile–water (85:15) with 10 mM-ammonium acetate and solvent B was acetonitrile–methanol–dichloroethane (85:10:5) with 10 mM-ammonium acetate. Gradient elution was performed at 2 ml/min using the following procedure: 100 % A for 3 min, 7 min linear gradient to 100 % B, 15 min hold and 2 min reverse gradient to 100 % A. Identities of βC isomers, retinol and retinyl esters were determined using HPLC-purified standards. Total liver VA stores were calculated by summing retinol and identifiable retinyl esters. For serum analysis in study 2, retinol isomers were separated using a Zorbax ODS C18 column (4·6 × 250 mm, 5 μm; Dupont) at 325 nm with an isocratic mobile phase of methanol–water–butanol (50:27:23) with 10 mM-ammonium acetate run at 1 ml/min. Identities of the retinol isomers were confirmed using the elution order determined by MacCrehan & Schönberger[15] and HPLC spectra. All tissue samples were run on a Waters HPLC system consisting of a Delta 600 quaternary HPLC pump, a 600 controller and a 2487 dual absorbance detector, and the samples were injected manually. C-23 alcohol was used as the internal standard, and standard curves were constructed for the quantification of carotenoids and retinoids.

Statistical analysis

Results are presented as means and standard deviations. Data were analysed using SAS software (version 8.2; SAS Institute). Outcomes of interest (gerbil body and liver weights, serum retinol, and liver retinol and carotenoids) were evaluated using a one-way ANOVA. Differences between groups were determined using the least significant difference test at α < 0·05. An arcsine-square-root transformation was used to homogenise variance expressed as percentages. Bioconversion factors of βC to retinol were calculated using differences in total liver VA in the βC-treated groups compared directly with the VA+ group after correction for the VA− group.

Results

Gerbil weights

In study 1, the final gerbil weights did not differ between the experimental groups (77·7 (SD 5·15) g) but were found to be higher than that of the baseline group (67·4 (SD 6·62) g, $P = 0·0043$). The final liver weights did not differ among the groups (3·05 (SD 0·42) g). In study 2, the final gerbil weights (68·8 (SD 5·63) g) and liver weights (2·47 (SD 0·35) g) were found to be similar among all the groups. Feed intakes did not differ within study 1 (6·42 (SD 1·07) g feed/d) or study 2 (5·05 (SD 0·69) g feed/d) (Table 2).
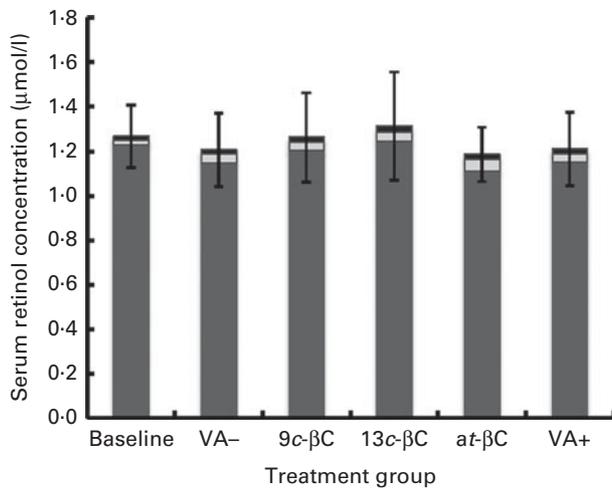
Serum retinol and carotenoids

In both studies, total serum retinol concentration did not differ among the treatment groups, and carotenoids were not detected. In study 1, the range of serum retinol concentrations was 1·43 (SD 0·12) μmol/l in the 13*c*-βC group to 1·70 (SD 0·18) μmol/l in the VA− group. In study 2, the range was found to be 1·19 (SD 0·12) μmol/l in the *at*-βC group to 1·27 (SD 0·14) μmol/l in the baseline group, and the relative ratio of isomers varied across the treatment groups (Fig. 1). Serum retinol concentrations differed between studies 1 and 2 ($P < 0·0001$). The composition of 9*c*-retinol in serum differed among the groups ($P = 0·032$). The proportion of 9*c*-retinol was similar among the 9*c*-βC, 13*c*-βC, *at*-βC and VA+ groups (1·94 (SD 0·50) %), and the proportion for the baseline and VA− groups (1·59 (SD 0·19) %) was found to be lower than that for the 13*c*-βC and *at*-βC groups (2·09 (SD 0·51) %), but

Table 2. Gerbil feed consumption in two studies administering low (15 nmol) and moderate (30 nmol) doses of β-carotene (βC) daily

(Mean values and standard deviations)

Treatment	*n*	Daily feed intake (g/d)	
		Mean	SD
Low βC intake			
9*c*-βC	10	5·83	1·15
13*c*-βC	10	6·66	0·73
at-βC	10	6·56	1·03
VA +	10	6·61	1·22
VA −	10	6·44	1·31
Moderate βC intake			
9*c*-βC	10	4·93	0·92
13*c*-βC	10	5·08	0·53
at-βC	10	4·86	0·86
VA +	10	4·93	0·25
VA −	10	5·53	1·16

c, cis; *at*, all-*trans*; VA, vitamin A.

Fig. 1. Serum retinol concentrations in Mongolian gerbils (*Meriones unguiculatus*) fed a vitamin A (VA)- and carotenoid-free feed at baseline after 4 weeks (study 2) of depletion (baseline, *n* 7), or treated with VA-free cottonseed oil (VA−), moderate doses of 30 nmol 9-*cis*-, 13-*cis*- or all-*trans* β-carotene (9*c*- ■, 13*c*- □ or a*t*-βC ■) in cottonseed oil, or retinyl acetate in cottonseed oil (VA+) for an additional 4 weeks. VA intake in the VA+ group was matched to one-half of the theoretical VA intake in the βC groups. Values are means (*n* 10), with standard deviations represented by vertical bars. The treatment groups did not differ significantly (*P*>0·05; ANOVA followed by least significant difference test).

did not differ from each other or the 9*c*-βC and VA+ groups (1·77 (SD 0·46)%). The composition of 13*c*-retinol did not differ among the groups (3·16 (SD 1·95)%). The composition of a*t*-retinol differed from baseline (96·7 (SD 0·50)%, *P*=0·021) in the 13*c*-βC and a*t*-βC groups, but did not differ from each other (94·1 (SD 1·94)%) or the VA−, 9*c*-βC and VA+ groups (95·1 (SD 1·76)%).
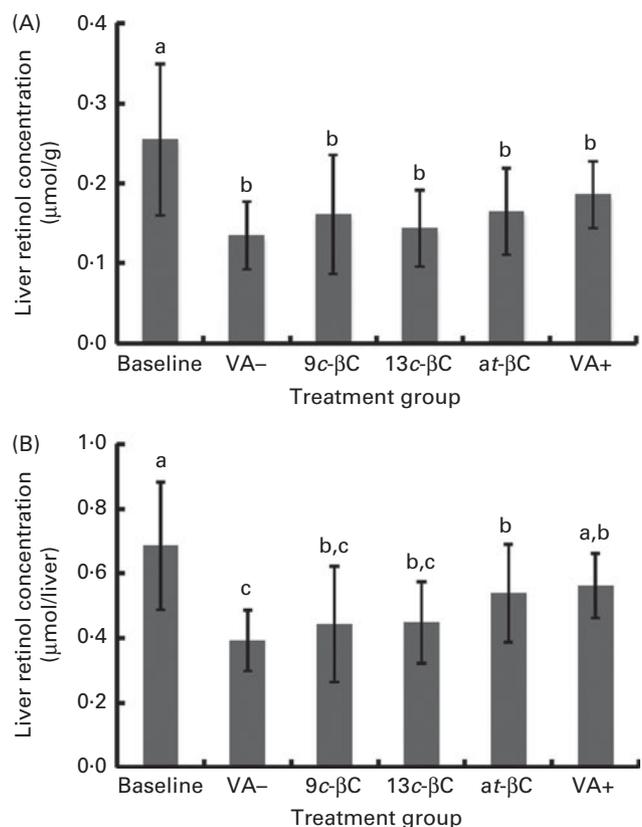
Liver retinol and carotenoids

In study 1, concentrations of liver retinol did not differ among the experimental groups, and the βC isomers did not maintain baseline liver stores in all the groups (*P*=0·006; Fig. 2(A)). Total liver retinol concentrations did not differ among the VA+, 9*c*-βC, 13*c*-βC and a*t*-βC groups, and the βC isomers failed to maintain baseline liver VA stores in all of these groups, except the VA+ group (*P*=0·0026; Fig. 2(B)). The 9*c*- and 13*c*-βC groups did not differ from the VA− group. In study 2, the βC isomers maintained baseline liver retinol status in all groups, except the VA− group, which did not differ from the 9*c*- and 13*c*-βC groups (*P*=0·0019; Fig. 3(A)). Total liver retinol stores were found to be highest in the VA+ group and lowest in the VA− group (*P*<0·0001; Fig. 3(B)). The VA− group did not differ from the 9*c*-βC group, but all the βC isomers resulted in similar total liver stores.

Carotenoids were not detected in the liver of the baseline, VA− and VA+ groups in either study. In studies 1 and 2, total liver βC concentration was found to be highest in the 9*c*-βC group followed by the a*t*-βC and 13*c*-βC groups (study 1, *P*<0·0001; study 2, *P*<0·0001; Table 3). The presence of the administered βC isomer and the other two isomers in the liver demonstrated *in vivo* isomerisation of
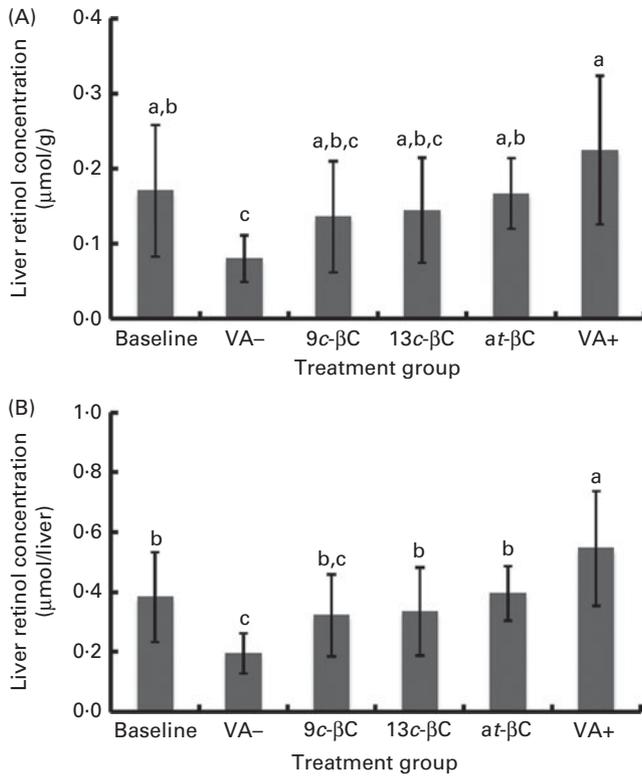
the βC doses, and the relative proportions of the βC isomers varied among these groups (Table 3).

Bioconversion factors and utilisation rates

Bioconversion factors for the βC-treated groups were calculated by determining the total liver VA stores above the negative control and by directly comparing this value to that of the VA+ group. Bioconversion factors ranged from 115 to 168% and 140 to 175% of the IOM's recommended values in studies 1 and 2, respectively (Table 4). Comparison of the 9*c*- and 13*c*-βC isomers with the a*t*-βC isomer resulted in mass equivalents of 2·9 and 1·6 µg 9*c*-βC and 2·6 and 1·4 µg 13*c*-βC to 1 µg a*t*-βC for studies 1 and 2, respectively. In study 1, the utilisation rate for the VA− group, which was deprived of VA for 21 d beyond baseline, was found to be 5·1 µg/100 g body weight and for the VA+ group, which was supplemented cumulatively with 84·6 µg retinol in the form of acetate, was 7·5 µg/100 g body weight. In study 2, the utilisation rate for the VA− group, which was deprived of VA for an additional 14 d in the

Fig. 2. Liver retinol (A) and total liver retinol (B) concentrations corrected for liver weight in Mongolian gerbils (*Meriones unguiculatus*) fed a vitamin A (VA)- and carotenoid-free feed at baseline after 3 weeks (study 1) of depletion (baseline, *n* 6), or treated with VA-free cottonseed oil (VA−), low doses of 15 nmol 9-*cis*-, 13-*cis*- or all-*trans* β-carotene (9*c*-, 13*c*- or a*t*-βC) in cottonseed oil, or retinyl acetate in cottonseed oil (VA+) for an additional 3 weeks. VA intake in the VA group was given at 1 µg retinol equivalent to 2 µg βC provided to the βC groups. Liver retinol was determined by pooling values for retinol and retinyl esters. Values are means (*n* 10), with standard deviations represented by vertical bars. a,b,c Mean values with unlike letters were significantly different (*P*<0·05; ANOVA followed by least significant difference test).

Fig. 3. Liver retinol (A) and total liver retinol (B) concentrations in Mongolian gerbils (*Meriones unguiculatus*) fed a vitamin A (VA)- and carotenoid-free feed at baseline after 4 weeks (study 2) of depletion (baseline, *n* 7), or treated with VA-free cottonseed oil (VA−), moderate doses of 30 nmol 9-*cis*-, 13-*cis*- or all-*trans* β-carotene (9*c*-, 13*c*- or a*t*-βC) in cottonseed oil, or retinyl acetate in cottonseed oil (VA+) for an additional 4 weeks. VA intake in the VA group was given at 1 µg retinol equivalent to 2 µg βC provided to the βC groups. Liver retinol was determined by pooling values for retinol and retinyl esters. Values are means (*n* 10), with standard deviations represented by vertical bars. ^{a,b,c} Mean values with unlike letters were significantly different ($P < 0.05$; ANOVA followed by least significant difference test).

Discussion

To assess the impact of thermal processing methods on plant foods, it is essential to determine the unique VA bioefficacies of *cis*-βC isomers at physiological levels. To our knowledge, no other studies have investigated the bioefficacies of βC isomers using amounts reflective of dietary intakes in animals with carotenoid metabolism similar to that of humans. In studies 1 and 2, the bioefficacies of 9*c*-, 13*c*- and a*t*-βC in oil at low (15 nmol) and moderate (30 nmol) doses were compared directly with preformed VA supplements. In study 1, the βC isomers did not maintain liver retinol stores; however, in study 2, at higher doses, all βC isomers maintained baseline liver stores and the VA supplement exceeded baseline VA status. Bioconversion factors were 15−75 % less efficient than the current reference values of the IOM. Factors for the supplemental *cis*-βC isomers were similar in each study, with midpoints of 6·4 and 5·3 µg *cis*-βC:1 µg retinol, in studies 1 and 2, respectively. Bioconversion factors for the supplemental a*t*-βC isomer were found to be 2·3 and 3·5 µg a*t*-βC:1 µg retinol in both studies, which are similar to the reported values of 2·5−4·6 in previous gerbil studies[10,13,14,16].

The utilisation rates within and between each study were not the same, but similar to those reported in previous gerbil studies[8,16]. For the VA− group, the utilisation rate in study 2 was almost half of that in study 1, indicating that the gerbils were conserving and recycling more retinol during the period of prolonged deprivation. The higher utilisation of the VA+ group in study 2 probably reflects the more concentrated preformed retinyl acetate dose that they received[17]. The βC doses failed to maintain baseline liver VA stores in study 1, suggesting that the utilisation rates of gerbils exceeded the amount of bioavailable provitamin A provided. Lee *et al.*[8] determined the VA utilisation rate of a VA-deplete adult gerbil to be 3·1 µg/100 g body weight. The difference in utilisation rates between study 1 (i.e. 5·1 µg/100 g body weight) and Lee *et al.*[8] may be due to the gerbils having a higher requirement as they were 16−26 d younger, or due to the variation in the level of

extended depletion and treatment periods, was found to be 2·7 µg/100 g body weight, and for the VA+ group, which received a total of 225·7 µg retinol, was 9·1 µg/100 g body weight.

Table 3. Total liver β-carotene (βC) and isomeric compositions in Mongolian gerbils (*Meriones unguiculatus*) administered with low (15 nmol) and moderate (30 nmol) doses of 9-*cis*-, 13-*cis*- or all-*trans* βC (9*c*-, 13*c*- or a*t*-βC)*

(Mean values and standard deviations; *n* 10 per βC group)

	Total liver βC (nmol)		9*c*-βC (%)		13*c*-βC (%)		a*t*-βC (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low βC intake								
9*c*-βC	7·3^a	1·3	68·6^a	4·6	10·2	0·9	21·3^c	3·8
13*c*-βC	2·4^c	0·46	39·3^b	5·6	12·3	4·2	48·4^b	4·4
a*t*-βC	4·9^b	2·0	31·2^c	13·0	12·6	2·8	56·2^a	10·7
P	<0·0001		<0·0001		NS		<0·0001	
Moderate βC intake								
9*c*-βC	5·0^a	0·6	21·6^c	4·1	6·2^b	1·2	72·2^a	3·4
13*c*-βC	2·2^c	0·5	57·1^b	6·0	9·6^a	1·8	33·2^b	6·6
a*t*-βC	3·2^b	0·5	69·6^a	3·6	9·1^a	2·9	21·3^c	2·3
P	<0·0001		<0·0001		0·0029		<0·0001	

^{a,b,c} Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

* The limit of detection for βC was <0·007 nmol.

Table 4. Bioconversion factors in Mongolian gerbils (*Meriones unguiculatus*) administered with low (15 nmol) or moderate (30 nmol) doses of β-carotene (βC)*

	μmol βC: 1 μmol retinol	μg βC: 1 μg retinol	IOM reference (μg βC:1 μg retinol)†	Percentage of IOM value	Percentage of at-βC bioefficacy‡
Low βC intake					
9c-βC	3·6	6·7	4·0	168	34
13c-βC	3·2	6·1	4·0	153	37
at-βC	1·2	2·3	2·0	115	100
Moderate βC intake					
9c-βC	3·0	5·6	4·0	140	63
13c-βC	2·7	5·0	4·0	125	70
at-βC	1·9	3·5	2·0	175	100

IOM, Institute of Medicine; *at*, all-*trans*; *c*, *cis*.

* *n* 10 per βC group.

† The IOM reference value for supplemental *cis*-βC isomers was based on the current retinol activity equivalents from food, which assumes a bioavailability that is equivalent to 50 % that of a*t*-βC for all *cis*-βC isomers.

‡ Determined by direct comparison with bioconversion factors for the a*t*-βC isomer in studies 1 and 2.

depletion[16,17]. In study 2, the depletion period was extended to reach deficiency, which resulted in a similar utilisation rate (i.e. 2·7 μg/100 g body weight) to that of the Lee *et al.*'s value, and doses were increased to allow for adequate VA storage in the βC-treated groups.

It is important to consider the impact of VA status on bioconversion factors. The utilisation rate slows and recycling becomes more efficient during the state of deficiency and the converse occurs when the status is sufficient[17,18]. This departure in utilisation rates between the VA-deplete and VA-replete groups can therefore lead to the inflation of conversion factors, which may partially explain the higher conversion factors in study 1 compared with study 2 for the *cis*-βC isomers. The VA− group had higher VA status in study 1 than in study 2 where the depletion and treatment periods were extended. Furthermore, bioconversion of βC to retinol in humans and gerbils is dependent on dose size[19,20] and is inversely related to VA status[21,22], which may explain the difference in bioconversion factors for the a*t*-βC isomer between the two studies because the dose was doubled in study 2.

In study 2, the relative VA values of the 9*c*- and 13*c*-βC isomers were similar with bioefficacies of 63 and 70 % that of the a*t*-βC isomer, respectively. In previous studies, the relative VA values of the 9*c*- and 13*c*-βC isomers compared with that of the a*t*-βC isomer were 33−61 and 48−74 %, respectively, in rats[23−25] and 38 and 62 %, respectively, in gerbils[7]. The variations in the VA values determined in the previous studies may be due to the differences in the amount (0·6−224·4 μg βC/d) and duration (7−28 d) of dosing, the depletion period (20−56 d) or the basis of calculation (i.e. body-weight gain *v.* liver VA)[7,23−25]. The gerbil provides a more advantageous model for studying carotenoid metabolism compared with the rat because, like humans, they are capable of cleaving provitamin A carotenoids to retinal and absorbing physiological doses intact[8,26,27]. Although rats are efficient cleavers of βC in the small intestine, they only absorb βC intact under conditions of great dietary excess[28−30]. The present study demonstrates more efficient conversion of the *cis*-βC isomers, particularly 9*c*-βC, in gerbils than that found by Deming *et al.*[7], which, again, is likely attributable to the larger dose size used in that study.

The observation of higher total liver βC concentration found in the 9*c*-βC group suggests that the 9*c*-βC isomer may be more efficiently delivered to the liver. *In vitro* studies have suggested that the *cis*-βC isomers are similarly or more efficiently incorporated into micelles during digestion[11,31−33], but data are inconsistent regarding the efficiency of *cis*-βC isomer uptake by intestinal cells[31−33]. Based on accumulation in human serum, the a*t*-βC isomer appears to be absorbed and/or packaged into the chylomicra more efficiently than the *cis*-βC isomers, with 13*c*-βC being the predominant *cis*-βC isomer[34−37]. Additionally, in human subjects fed supplements or *Dunaliella bardawil* containing an a*t*-βC:9*c*-βC ratio of approximately 50:50, the a*t*-βC isomer appears to be preferentially incorporated into the chylomicra and VLDL[38−40]. However, *in vivo* interconversion of βC isomers, documented previously in human subjects[41] and gerbils[37] and confirmed in the present study, may have a positive impact on the VA values of the *cis*-βC isomers, and result in inaccurate interpretations of their bioavailabilities when confirmed solely by the presence of the isomer of interest. More work is necessary to elucidate common *in vivo* patterns for and mechanisms of βC isomer interconversion as none is apparent from the isomeric composition found in the gerbil liver in these studies. Furthermore, enzymatic isomerisation of *cis*-βC isomers to a*t*-retinal by β-carotene 15,15′-mono-oxygenase 1 was observed in rats, with 9*c*-βC converting to a mixture of 9*c*-, 13*c*- and a*t*-retinal and 13*c*- and a*t*-βC converting primarily to a*t*-retinal[42]. In a previous study, the composition of retinol isomers in the liver of gerbils was determined to be similar among groups dosed with βC isomers and the negative control, with *cis*-βC isomers constituting approximately 10 % of total liver VA[37]. Similarly, the isomeric composition of serum retinol in study 2 was found to be 3·3−6·5 % of *cis*-retinol isomers, which supports the *in vivo* isomerisation of βC and/or retinol. Together, these findings demonstrate that the isomeric compositions of liver and serum retinol do not reflect the dietary intake of βC, and that the a*t*-retinol isomer appears to be preferable for the storage and circulation of VA.

The lack of the difference observed in liver VA stores among the 9*c*-, 13*c*- and a*t*-βC groups and bioefficacies of the *cis*-βC isomers >60 % that of the a*t*-βC isomer contradicts the idea that the *cis:trans* ratio significantly decreases the VA value of

plant foods. These findings support the conclusion made by Howe *et al.*[10] that regular, moderate intakes of *cis*-βC isomers from plant foods allow for efficient absorption and bioconversion to VA in gerbils. The cassava feeds used by Howe *et al.* contained 41–47 % of *cis*-βC isomers; by using a bioefficacy of *cis*-βC isomers equivalent to two-thirds that of the *at*-βC isomer, the VA value may have been decreased by only 14–16 %. In fact, Howe *et al.*[10] demonstrated a conversion factor of 3·7 µg βC:1 µg retinol, which is similar to the values from plant foods containing much lower concentrations of *cis*-βC isomers[43,44]. Similar bioconversion factors for thermally processed provitamin A-biofortified cassava have recently been determined in women, ranging from 4·2–4·5 µg βC:1 µg retinol depending on oil content[45]. Additionally, the matrix of certain plant foods can have a negative impact on the VA value[46], in which case thermal processing can increase βC bioavailability[33,47,48]. It is therefore important for nutritionists and plant breeders to holistically consider the impacts of processing on matrix effects and carotenoid concentrations when establishing provitamin A biofortification targets in staple crops.

Liver is the primary storage site of retinol in mammals and is considered the best measure of VA status[18]. Serum retinol concentrations do not provide an effective measure of status because of homeostatic regulation over a wide range of liver stores[18], as demonstrated by similar concentrations among all the treatment groups within both studies. In that regard, the difference between serum retinol concentrations between studies 1 and 2 is interesting, considering that the gerbils in study 2 received twice the supplemental doses and liver VA concentrations did not differ between the two studies. Subclinical deficiency has recently been defined as liver stores < 0·1 µmol retinol/g liver[18]; according to this definition, the VA− group in study 1 was nearing deficiency and the VA− group in study 2 was deficient, but serum retinol concentrations were not low (i.e. concentrations were > 0·7 µmol/l). Additionally, the absence of carotenoids in serum is expected because the lipid profile of gerbils differs from that of humans in that the major circulating lipoprotein is HDL and carotenoids are predominantly associated with LDL during fasting[49].

In summary, the 9*c*-, 13*c*- and *at*-βC isomers prevented retinol deficiency in gerbils when provided in amounts reflective of moderate dietary intake, and the *cis*-βC isomers demonstrated bioefficacies >60 % that of the *at*-βC isomer when baseline liver stores were maintained. The results of these studies suggest that mild thermal processing modestly decreases the VA value of plant foods through the generation of the *cis*-βC isomers. Furthermore, the currently assigned bioavailability of the *cis*-βC isomers, equivalent to 50 % that of the *at*-βC isomer[4], may need to be clarified as being dependent on dose size and VA status. More research is needed to elucidate the mechanism of *in vivo* isomerisation of *cis*-βC to *at*-βC and to assess the bioavailabilities of *cis*-βC isomers from plant foods in human subjects.

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