Cassava with enhanced β-carotene maintains adequate vitamin A status in Mongolian gerbils (*Meriones unguiculatus*) despite substantial *cis*-isomer content

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Efforts to increase β-carotene in cassava have been successful, but the ability of high-β-carotene cassava to prevent vitamin A deficiency has not been determined. Two studies investigated the bioefficacy of provitamin A in cassava and compared the effects of carotenoid content and variety on vitamin A status in vitamin A-depleted Mongolian gerbils (*Meriones unguiculatus*). Gerbils were fed a vitamin A-free diet 4 weeks prior to treatment. In Expt 1, treatments (ten gerbils per group) included 45 % high-β-carotene cassava, β-carotene and vitamin A supplements (intake matched to high-β-carotene cassava group), and oil control. In Expt 2, gerbils were fed cassava feeds with 1·8 or 4·3 nmol provitamin A/g prepared with two varieties. Gerbils were killed after 4 weeks. For Expt 1, liver vitamin A was higher ($P<0.05$) in the vitamin A (1·45 (SD 0·23) μmol/liver), lower in the control (0·43 (SD 0·10) μmol/liver), but did not differ from the β-carotene group (0·77 (SD 0·12) μmol/liver) when compared with the high-β-carotene cassava group (0·69 (SD 0·20) μmol/liver). The bioconversion factor was 3·7 μg β-carotene to 1 μg retinol (2 mol:1 mol), despite 48 % *cis*-β-carotene [(*Z*)-β-carotene] composition in cassava. In Expt 2, cassava feed with 4·3 nmol provitamin A/g maintained vitamin A status. No effect of cassava variety was observed. Serum retinol concentrations did not differ. β-Carotene was detected in livers of gerbils receiving cassava and supplements, but the *cis*-to-*trans* ratio in liver differed from intake. Biofortified cassava adequately maintained vitamin A status and was as efficacious as β-carotene supplementation in the gerbil model.

***cis*-β-Carotene: *trans*-β-Carotene: (*Z*)-β-Carotene: (*E*)-β-carotene: Cassava: Vitamin A**

Vitamin A deficiency is a major health problem particularly in Africa and South-East Asia where foods rich in preformed vitamin A (e.g. milk, eggs and liver) or provitamin A (e.g. carrots, sweet potato and pumpkin) are lacking. Deficiencies can result in blindness, night blindness, decreased immunity, and increased morbidity and mortality. Due to the essential role of vitamin A in reproduction and growth, women and children are particularly affected.

Cassava is a staple food for many populations at risk for vitamin A deficiency, especially in Africa. In cassava, β-carotene is the primary provitamin A carotenoid, but concentrations in typical white cassava are low, about 1 μg/g fresh weight or about 3 μg/g dry weight[1,2]. Ongoing efforts to breed cassava for increased provitamin A have identified genotypes with more than 10 μg β-carotene/g fresh weight[2]. These concentrations are still low compared to typical carrot, i.e. 130 μg β-carotene/g fresh weight[3]. Low predicted bioconversion rates of β-carotene to vitamin A (i.e. 12 μg to 1 μg all-*trans* retinol (i.e. all-(*E*)-retinol) proposed by the Institute of Medicine[4]) and generally poor bioavailability of provitamin A carotenoids from food[4,5] further contribute to questions regarding the bioefficacy of biofortified cassava.

Bioavailability of provitamin A carotenoids from foods is not well understood. Before breeding efforts continue, it is essential to assess whether carotenoid-biofortified cassava can positively contribute to vitamin A status. Many factors influence carotenoid absorption and bioconversion[6]. Lack of methodology to directly measure absolute bioavailability further complicates the issue. Measuring change in serum carotenoid concentrations following intervention has been used[5]. Results from this approach are affected by carotenoid and vitamin A regulation in the blood and bioconversion of provitamin A to vitamin A preceding entry into the bloodstream[7]. Serum carotenoid assessment is also influenced by the amount of carotenoid administered in the dose or meal, presence of adequate fat during adsorption, and vitamin A status due to its regulation of the conversion of β-carotene to vitamin A[8–11].

Other methods have been used to evaluate provitamin A bioavailability. The most common methods include *in vitro* intestinal cell methods[12–15], stable isotope tracers[16,17] and animal models[18–21]. *In vitro* models using Caco-2 cells best model bioaccessibility and do not reflect enzymatic regulation of bioconversion. Isotope tracer studies in man are perhaps the best method[17], but many experimental factors such as diet and

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vitamin A status are difficult to control. Appropriate animal models provide a lower-cost alternative with greater experimental control. In addition, the use of animal models permits direct measurement of liver vitamin A, which is considered the best indicator of vitamin A status[22], and allows calculation of true bioefficacy. For carotenoids, rats and mice are not appropriate because unlike man, they absorb very little β-carotene intact[18–20]. In contrast, the Mongolian gerbil absorbs and metabolises β-carotene similarly to man[21,23–26].

The objective of the present research was to investigate the bioefficacy of β-carotene from biofortified cassava in Mongolian gerbils with depleted vitamin A status. The high *cis*-β-carotene (i.e. (Z)-β-carotene) content of processed cassava provided an opportunity to examine the effect of the *cis* isomer on bioconversion to vitamin A. Two studies were conducted in parallel to compare the bioefficacy of β-carotene from cassava with vitamin A and β-carotene supplements (Expt 1) and to investigate the effect of dietary level and cassava variety on vitamin A status (Expt 2).

Materials and methods

Cassava and feeds

Three cassava varieties (white, #1 and #2) were crated and shipped from the International Institute of Tropical Agriculture in Nigeria. Upon arrival, damaged tubers were discarded, and remaining tubers were peeled, cut into approximately 2–3 cm slices, boiled for 30 min, cooled and frozen at −20°C. The cassava varieties used were low in cyanogenic compounds and ranged from 13 to 45 mg cyanide equivalents/100 g fresh weight, which were removed by boiling sliced tubers in a large amount of water. Frozen cassava was then freeze-dried and ground into a powder with a coffee grinder. High β-carotene cassava varieties were stored at −80°C and white cassava was stored at −20°C. All varieties were analysed for carotenoid concentration (Table 1) and used to prepare seven powdered Mongolian gerbil feeds with variable cassava (Table 2) and carotenoid compositions (Table 3).

With the assistance of Harlan-Teklad (Madison, WI, USA), gerbil feeds were designed to use cassava as the carbohydrate source. For the vitamin A-depletion phase, the vitamin A- and carotenoid-free feed was 30% white cassava (Table 2). For the treatment phase of the first experiment, feeds were 45% white or cassava #1. For the second experiment, feeds were 40 and 17% cassava #1 and 35 and 15% cassava #2 (Table 2). High and low cassava percentages were designed to equalise the β-carotene concentrations from cassava #1 and #2. Differences in the percentage cassava among feeds were offset with sucrose–starch (2:1). Because synthetic vitamin A and provitamin A carotenoids were not added to the feeds, the only source of vitamin A was from the cassava.

Table 1. Carotenoid concentrations (nmol/g dry weight cassava) in three varieties of cassava

(Mean values and standard deviations)

Cassava variety	α-Carotene		*trans*-β-Carotene		9-*cis*-β-Carotene		13-*cis*-β-Carotene		Total theoretical retinol*
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
White	0·01	0·001	0·37	0·02	0·01	0·001	0·19	0·001	1·15
#1	0·32	0·02	5·68	0·40	1·68	0·22	2·59	0·21	20·2
#2	0·44	0·01	5·87	0·12	2·72	0·36	2·79	0·06	23·2

* Theoretical vitamin A assumes 1 mol β-carotene provides 2 mol retinol and 1 mol α-carotene provides 1 mol retinol.

Table 2. Composition of experimental feeds (g/kg feed) fed to Mongolian gerbils (*Meriones unguiculatus*) differing by cassava content*

	Cassava content (%)					
	45	40	35	30	17	15
Cassava	450	403	350	300	172	150
Vitamin-free casein	363	363	363	363	363	363
L-Cystine	5·45	5·45	5·45	5·45	5·45	5·45
Sucrose	219·0	250·3	285·7	320	404·3	419·0
Maize starch	110·0	125·7	143·3	160	202·7	230·0
Cottonseed oil	109·0	109·0	109·0	109·0	109·0	109·0
Cellulose	109·0	109·0	109·0	109·0	109·0	109·0
Mineral mix†	63·64	63·64	63·64	63·64	63·64	63·64
MgO	3·18	3·18	3·18	3·18	3·18	3·18
Vitamin mix‡	9·1	9·1	9·1	9·1	9·1	9·1
Vitamin D	0·0008	0·0008	0·0008	0·0008	0·0008	0·0008
Vitamin E	0·44	0·44	0·44	0·44	0·44	0·44
Choline bitartrate	4·55	4·55	4·55	4·55	4·55	4·55

* Provided by Harlan-Teklad, Madison, WI, USA.
† AIN-93M-MX[27].
‡ Vitamin mix provided the following (mg/kg feed): biotin, 0·4; calcium panthothenate, 66·1; folic acid, 2; inositol, 110·1; menadione, 49·6; niacin, 99·1; *p*-aminobenzoic acid, 110·1; pyridoxine-HCl, 22; riboflavin, 22; thiamin-HCl, 22; vitamin B$_{12}$ (0·1% in mannitol), 29·7; ascorbic acid (97·5%), 1016·6.

Table 3. Treatment groups, carotenoid concentrations and theoretical daily retinol intake for two studies performed in Mongolian gerbils (*Meriones unguiculatus*)*

(Mean values and standard deviations)

Cassava in feed (%)	Dose	α-Carotene (nmol/g feed)		trans-β-Carotene (nmol/g feed)		9-cis-β-Carotene (nmol/g feed)		13-cis-β-Carotene (nmol/g feed)		Theoretical retinol intake (nmol/d)†
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Expt 1										
45% white	Oil	0·01	0·004	0·15	0·01	0·04	0·01	0·06	0·01	3·0
45% #1	Oil	0·14	0·003	2·74	0·06	1·28	0·03	1·21	0·03	62·6
45% white	βC	0·01	0·004	0·15	0·01	0·04	0·01	0·06	0·01	64·6
45% white	VA	0·01	0·004	0·15	0·01	0·04	0·01	0·06	0·01	64·6
Expt 2										
40% #1	–	0·13	0·02	2·47	0·11	0·75	0·05	1·07	0·06	55·7
35% #2	–	0·18	0·004	2·11	0·07	0·96	0·06	0·94	0·03	52·6
17% #1	–	0·06	0·003	1·01	0·03	0·28	0·03	0·42	0·002	22·2
15% #2	–	0·08	0·02	0·97	0·03	0·44	0·05	0·43	0·02	24·1

βC, β-carotene; VA, vitamin A.

* For details of procedures and diets, see the Materials and methods section and Tables 1 and 2.

† Theoretical retinol intakes for 45% cassava #1 group in Expt 1 are calculated from actual feed consumption and carotenoid composition over 28 d. Intakes for other treatment groups in Expt 1 represent the mean daily provitamin A dose supplied to the animal. Doses were equalised daily based on mean feed consumption by the 45% cassava #1 group (approximately 5·9 g/d). The contribution of provitamin A in white cassava, 3 nmol/d, was added to daily theoretical retinol intake in feeds with white cassava. In Expt 2, theoretical retinol intakes were calculated based on a mean feed consumption of 6·4 g/d. All calculations assume 100% bioefficacy of provitamin A, i.e. 1 mol β-carotene provides 2 mol retinol and 1 mol α-carotene provides 1 mol retinol.

Feeds were stored at −20°C to prevent carotenoid degradation during the treatment phase.

Carotenoid composition of cassava and feeds

Cassava and feeds were analysed for carotenoid concentrations (Tables 1 and 3) according to published procedures[28]. Because this method was developed for maize and required saponification at 85°C, variations of the method were performed to verify the use of high temperature and saponification on carotenoid extraction. Results showed no difference in extracted carotenoids saponified at 60°C compared with 85°C, but 5–10% less carotenoid was extracted without saponification (at room temperature and 60°C) and with saponification at room temperature. Analysis of extracted carotenoids was adapted from published procedures[28–30]. A Waters HPLC system (Waters Corporation, Milford, MA, USA) consisting of a guard column, C30 YMC carotenoid column (4·6 × 250 mm, 3 μm), 1525 binary HPLC pump, 717 autosampler, and either a 996 or 2996 photodiode array detector was used. Solvent A consisted of methanol–water (92:8, v/v) with 10 mM-ammonium acetate. Solvent B was 100% methyl-tertiary-butyl ether. Gradient elution was performed at 1 ml/min with a 30 min linear gradient from 70 to 40% A. Positive identification of lutein, zeaxanthin, β-cryptoxanthin and β-carotene was determined using purified standards and absorption spectra. Chromatograms were generated at 450 nm.

Animals and procedures

Male 40 d old Mongolian gerbils (*n* 87) were obtained from Charles River Laboratories (Kingston, NY, USA). Gerbils were individually housed in plastic cages and given free access to food and water. Gerbils were weighed daily and monitored for health until all were thriving, at which time, they were weighed every 2 d. Three gerbils died during the first 2 weeks due to self-injury or unwillingness to adapt to depletion feed. After the 4-week depletion phase, six gerbils were killed at baseline. Remaining gerbils were sorted into weight-matched treatment groups (nine or ten per group) and placed on their respective feeds. After 4 weeks, gerbils were killed by exsanguination through direct cardiac puncture while under isoflurane anaesthesia. Blood samples were centrifuged (2200 *g*) for 15 min in BD Vacutainer™ Gel and Clot Activator tubes (Becton Dickinson; Franklin Lakes, NJ, USA) for serum isolation. Livers were excised and stored at −80°C until vitamin A and carotenoid analysis. All animal handling procedures were approved by University of Wisconsin-Madison's Research Animal Resource Center.

Experimental design

Expt 1. Dietary treatment groups included 45% cassava #1 dosed with cottonseed oil, 45% white cassava supplemented with β-carotene in oil, 45% white cassava supplemented with vitamin A in oil, and 45% white cassava dosed with oil as a negative control (Table 3). Vitamin A (as retinyl acetate) and β-carotene in oil doses were equalised to the total daily provitamin A consumption of the 45% cassava #1 group assuming that 1 mol β-carotene provides 2 mol vitamin A and 1 mol α-carotene provides 1 mol vitamin A (i.e. 100% bioefficacy). Dosing was performed twice daily approximately 5 h apart to expand the absorption period for vitamin A and β-carotene.

Expt 2. Treatment groups received either 1·8 nmol provitamin A/g feed from 17% cassava #1 and 15% cassava #2 or 4·3 nmol provitamin A/g feed from 40% cassava #1 and 35% cassava #2 (Table 3).

Preparation of β-carotene and vitamin A supplements for Expt 1

Oil doses were prepared by dissolving a β-carotene supplement (GNC Inc., Pittsburg, PA, USA) or retinyl acetate (Sigma, St Louis, MO, USA) into cottonseed oil using

sonication. Purity of supplements were determined to be >95 % all-*trans*-β-carotene and >99 % all-*trans*-retinyl acetate. Final concentrations of β-carotene and vitamin A in oil were determined by dissolving an aliquot in hexanes and calculating the concentration using the $E_{1cm}^{1\%}$ (2592 for β-carotene and 1845 for vitamin A) at 450 and 325 nm, respectively. The oil doses delivered 0·405 nmol β-carotene/μl and 0·795 nmol vitamin A/μl.

Serum and liver preparation for HPLC

All samples were analysed under gold fluorescent lights to prevent photo-oxidation and isomerisation. Retinyl butyrate (31 μM in methanol) was synthesised and added as an internal standard to determine extraction efficiency in serum (92 (SD 8) %) and liver (88 (SD 13) %). It was also used externally for quantification of retinol and retinyl esters. Modified published procedures were used for vitamin A and β-carotene analysis of serum and liver[31–33]. Serum (500 μl) was extracted three times with hexane (1 ml) and dried under argon. Liver (0·7–0·9 g) was ground with approximately 3–5 g anhydrous sodium sulphate, extracted repeatedly with dichloromethane, and filtered into a 50 ml volumetric flask. An aliquot (5 ml) of the liver extract was dried under argon. Dried serum and liver samples were reconstituted in 100 μl methanol–dichloroethane (50:50, v/v) and injected (50 μl) into the HPLC system described previously using a Resolve™ C18 column (5 μm, 3·9 × 300 mm; Waters Corporation, Milford, MA, USA). Total liver vitamin A reserves were calculated by summing retinol and all identifiable retinyl esters using photo-diode array detection.

Statistical analysis and calculations

Data were analysed using Minitab 15.1.0 (Minitab Inc., State College, PA, USA). Outcomes of interest including gerbil weights, serum retinol concentration, and liver vitamin A and β-carotene content and concentrations were evaluated using ANOVA at $\alpha < 0.05$. Differences between treatment groups were determined using least significant differences at $\alpha < 0.05$.

Results

Carotenoid concentration of feeds and feed consumption

The 30 % white cassava feed used for the vitamin A depletion phase of the experiments contained 0·17 nmol β-carotene/g. β-Carotene concentrations in the treatment feeds ranged from 0·25 (SD 0·01) in the white cassava feed to 5·23 (SD 0·06) nmol/g feed in the 45 % cassava #1 group (Table 3). Feed intake during the treatment phase did not differ among groups ($P = 0.43$) and was 5·9 (SD 1·2) and 6·4 (SD 1·7) g/d for Expt 1 and Expt 2, respectively.

Gerbil weights

Gerbils in the baseline group (*n* 6) weighed 66·3 (SD 5·1) g at 4 weeks. One gerbil injured himself and was euthanised 1·5 d prior to kill, but was included in all analyses except serum retinol. Gerbil weight gain began to plateau at approximately

5 weeks. For all treatment groups, final gerbil weights did not differ ($P = 0.32$) and ranged from 72·2 (SD 4·9) to 74·6 (SD 4·7) g in the 45 % white cassava and 45 % cassava #1 groups, respectively.

Serum and liver vitamin A and carotenoid concentrations

Serum retinol concentrations (1·35 (SD 0·20) μmol/l) did not differ among treatment groups whether the studies were considered alone ($P = 0.95$ Expt 1 and $P = 0.70$ Expt 2) or combined ($P = 0.98$) and ranged from 1·30 (SD 0·13) to 1·39 (SD 0·28) μmol/l in the 40 % cassava #1 and oil control groups, respectively. No carotenoids were detected in the serum.

Expt 1. As expected, total hepatic vitamin A was greater in the vitamin A supplement group compared with the other groups (Fig. 1 (A); $P < 0.001$). Total hepatic vitamin A in the 45 % cassava #1 and β-carotene supplement groups did not differ ($P = 0.82$) and was approximately half of the vitamin A supplement group. Total hepatic vitamin A in the 45 % cassava #1 ($P = 0.009$), β-carotene ($P = 0.004$) and vitamin A ($P < 0.001$) groups was higher than the control due to continued vitamin A depletion during the treatment phase. The baseline group did not differ from 45 % cassava #1, β-carotene or control groups. Differences between groups were similar on a liver concentration basis, except that the 45 % cassava #1 group did not differ from the control (Fig. 1 (B); $P = 0.096$).

Both *cis*- and *trans*-β-carotene were measured in the liver of gerbils fed β-carotene from cassava or β-carotene supplements (Fig. 1 (C)). The total and *trans*-β-carotene content did not differ between the two groups ($P = 0.75$ and $P = 0.87$, respectively), but *cis*-β-carotene was greater in the β-carotene group than the 45 % cassava #1 group ($P = 0.044$). Results on a concentration basis are not shown, but were similar to total liver content.

Retinol conversion factors were calculated for Expt 1. Bioconversion of β-carotene to vitamin A was calculated using the stored hepatic vitamin A (total liver vitamin A of treatment group minus control group). Conversion factors were 3·7 μg β-carotene to 1 μg retinol (3·7:1 (2·0:1 on a molar basis)) for the 45 % cassava #1 group and 2·8:1 (1·5:1 on a molar basis) for the β-carotene supplement group. Similar conversion factors were obtained when calculated as net intake of provitamin A as β-carotene divided by the sum of liver storage and use (estimated from the difference of the baseline and control groups).

Percentage liver vitamin A storage was calculated by subtracting the hepatic vitamin A content of the control group from each treatment group and dividing by the vitamin A intake as theoretical retinol minus the intake of the control group. Contribution of liver β-carotene was ignored due to its low content compared with vitamin A. Percentage liver vitamin A storage did not differ between the 45 % cassava #1 and β-carotene supplement groups (16 and 20 %, respectively). The vitamin A group had the highest percentage liver storage (59 %), which was greater than any other treatment group ($P < 0.001$).

Expt 2. Total hepatic vitamin A did not differ among cassava or baseline groups, although gerbils receiving lower β-carotene had lower hepatic vitamin A (Fig. 2 (A)). Gerbils receiving the 35–40 % cassava had greater total hepatic
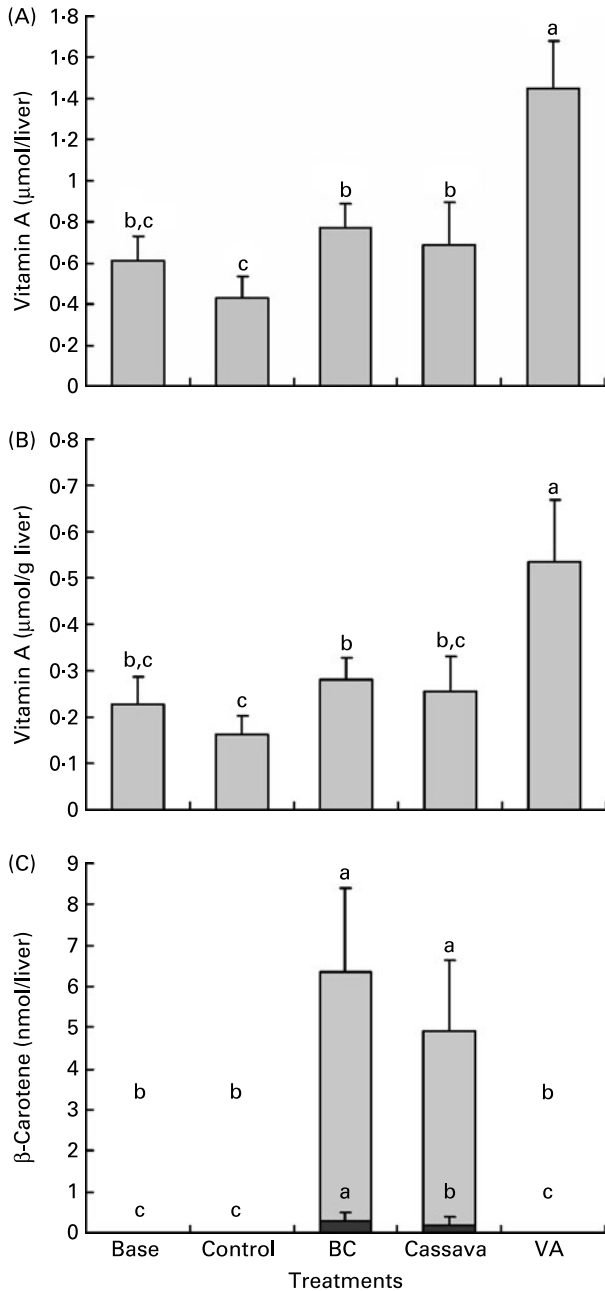
Fig. 1. Total liver vitamin A (A), liver vitamin A concentrations (B) and total β-carotene per liver (C) of vitamin A-depleted Mongolian gerbils (*Meriones unguiculatus*) at baseline (Base) and after 4 weeks of treatment including: 45 % white cassava feed with oil (Control), 45 % white cassava with β-carotene in oil (BC), 45 % high-β-carotene cassava #1 feed with oil (Cassava) and 45 % white cassava with vitamin A in oil (VA) from Expt 1. β-Carotene and vitamin A doses were equalised to the β-carotene consumption in the 45 % high-β-carotene cassava group on the prior day assuming 100 % bioconversion of β-carotene to retinol. Doses were divided by two and administered 5 h apart. Values are means with standard deviations depicted by vertical bars (*n* 10 for treatment groups and *n* 6 for baseline). [a,b] Mean values with unlike letters were significantly different (*P*<0·05). (C), Upper letters represent differences of total and *trans*-β-carotene (□) and lower letters represent differences of *cis*-β-carotene (■).

vitamin A than gerbils in the control group (*P* = 0·017 and 0·001, respectively), but did not differ with other treatment groups or the baseline group. Hepatic vitamin A content of gerbils receiving 15–17 % cassava feeds did not differ from
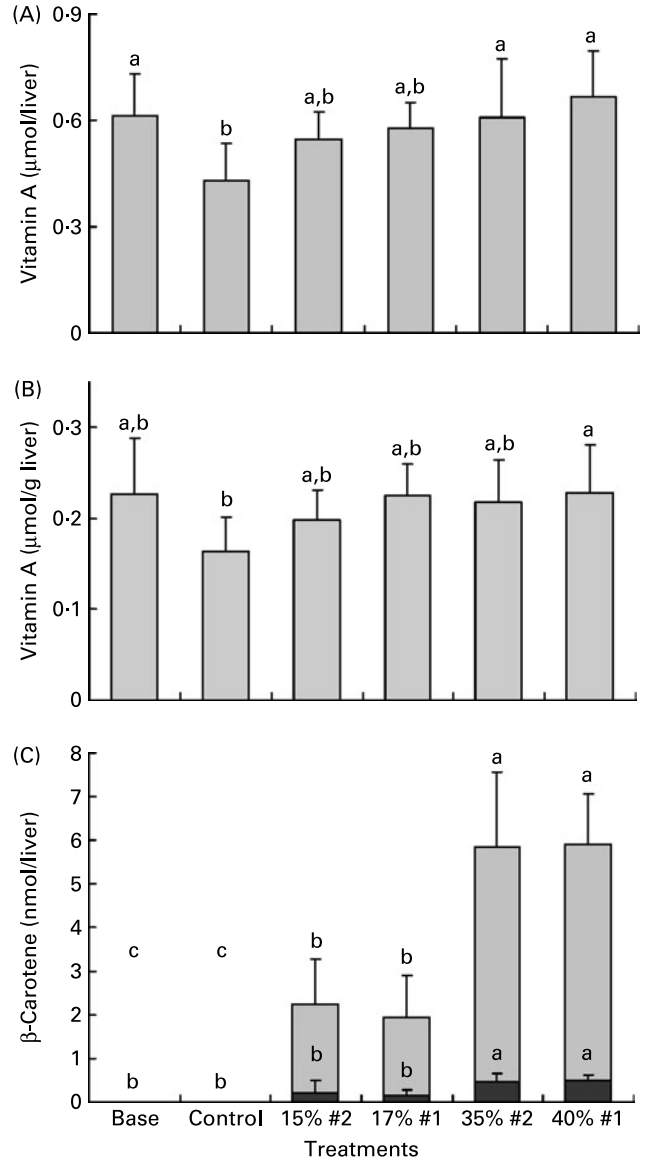

Fig. 2. Total liver vitamin A (A), liver vitamin A concentrations (B) and total β-carotene (C) of vitamin A-depleted Mongolian gerbils (*Meriones unguiculatus*) at baseline (Base) and after 4 weeks of cassava treatment including 45 % white cassava (Control), 15 % cassava #2 (15 % #2), 17 % cassava #1 (17 % #1), 35 % cassava #2 (35 % #2) or 40 % cassava #1 (40 % #1) feeds from Expt 2. Values are means with standard deviations depicted by vertical bars (*n* 7–10). [a,b] Mean values with unlike letters were significantly different (*P*<0·05). (C), Upper letters represent differences of total and *trans*-β-carotene (□) and lower letters represent differences of *cis*-β-carotene (■).

control or baseline groups. Hepatic vitamin A concentrations did not differ among cassava and baseline groups. The 40 % cassava group had higher hepatic vitamin A concentrations than the control group (*P* = 0·027). All gerbils were considered to have a sufficient vitamin A status, defined as >0·07 μmol/g liver[34].

Total hepatic β-carotene (*cis*, *trans* and total) was higher in groups receiving 35–40 % cassava (*P*<0·001; Fig. 2 (C)). Hepatic total and *trans*-β-carotene in gerbils receiving 15–17 % cassava feeds was greater than baseline and control groups (*P*<0·001), but *cis*-β-carotene did not differ from baseline or control groups (*P*>0·15). Results for *cis*-, *trans*- and

total β-carotene concentrations were consistent with total content and are not shown. No effect of cassava variety was observed for liver vitamin A or β-carotene. Percentage liver vitamin A storage was calculated for the combined 15–17 % and 35–40 % treatment groups because varieties were fed at equivalent provitamin A concentrations and did not differ. Percentage liver vitamin A storage differed between the 15–17 % cassava and 35–40 % cassava and was 24 and 15 %, respectively ($P = 0.02$).

Discussion

Cassava is consumed through a variety of snacks and main dishes[35], and with enhanced β-carotene, it has the potential to positively alter vitamin A status. To evaluate bioefficacy, β-carotene from biofortified cassava was compared with β-carotene and vitamin A supplements in vitamin A-depleted gerbils. The percentages of cassava used in these studies (15–45 %) encompass the contribution of staple foods to typical diets of developing countries, as well as providing a range of provitamin A intakes for the gerbils. The cassava was peeled and boiled to remove cyanogenic compounds similarly to many human food preparation methods[35]. Processing is known to increase the percentage of cis-β-carotene[36–38], and in the present experiment the percentage increased from approximately 25 to 48 %, which is similar to the 30–52 % range observed for ten genotypes of processed cassava[38]. Thus, the provitamin A content of gerbil feeds and ratio of cis-to-trans β-carotene did not vary substantially from human food preparations.

Expt 1 demonstrates that equivalent vitamin A status can be achieved from consuming cassava or supplementation with small daily amounts of β-carotene. Gerbils in the high-β-carotene cassava and β-carotene supplement groups were able to maintain or improve their vitamin A status over the 4-week treatment period compared to baseline and control groups. The calculated conversion factor for β-carotene dissolved in oil was 2·8 μg β-carotene to 1 μg retinol (2·8:1), which was consistent with values determined in prior gerbil studies conducted in our laboratory, i.e. 2·4–2·9:1[31,32,39]. Reported conversion factors for bioefficacy in man range from 938:1 in healthy American males[34] to 2·4–2·7:1 in Indonesian children with marginal vitamin A status[8,34,40]. Conversion factors determined using vitamin A-depleted gerbils are most similar to factors determined in human subjects with low or depleted status, i.e. 2:1–3·8:1[8,41,42]. In a review of studies determining the bioequivalence of β-carotene in man, Thurnham[34] concluded that bioefficacy is greater when obtained using small amounts and in subjects with low vitamin A status. Gerbils show similarity in β-carotene metabolism to man and are less prone to the problems with human studies associated with assessment of vitamin A status, dietary provitamin A intake and health status (e.g. incidence of helminthes, subclinical infection, inflammation). Comparisons between human studies are further complicated due to effects of dose size and frequency, food preparation, food matrix and fat intake.

The bioconversion factor for provitamin A to vitamin A in cassava was 3·7:1. This factor is consistent with 3·5:1 determined for high-lycopene carrots fed at similar daily rates[43]. Another study found much larger bioconversion ratios with

a variety of carrots (i.e. typical orange, purple and high-β-carotene orange) ranging from 13 to 30:1[33], but daily provitamin A intake was 5- to 25-fold higher and the depletion period was 3 weeks shorter than the cassava and high-lycopene carrot studies. Bioconversion factors for maize range from 2·1 to 3·3:1 in high-β-carotene, high-β-cryptoxanthin and high-lutein and zeaxanthin maizes[31,39,44]. Although the conversion factor for high-β-carotene maize in one study was determined on an all-trans-β-carotene basis instead of a total provitamin A basis[31], the conversion factors are similar to cassava in a vitamin A-depleted model.

Bioefficacy of cis-β-carotene is generally accepted to be less than the trans isomer, but this is based on few studies. Reported vitamin A values determined in gerbil and rat models range from 23 to 61 % for 9-cis-β-carotene and from 48 to 74 % for 13-cis-β-carotene[45]. However, bioconversion factors were similar for gerbils on cassava and high-lycopene carrot feeds with similar total β-carotene content, but different cis content, 48 and 2·5 %, respectively[43]. In the current study, cassava feeds contained 48 % cis-β-carotene and the supplement was <4 %, and yet cis-β-carotene in the liver was 3–8 % regardless of treatment group. Therefore, no relationship between hepatic cis-β-carotene and dietary intake was observed. In the β-carotene supplement group, presence of cis isomers could be attributed to an artifact of analysis or to trans-to-cis isomerisation occurring in the body. The lack of hepatic cis isomers in gerbils receiving cassava may indicate poor absorption and utilisation[38,46], cis-to-trans isomerisation within the body[47] or preferential conversion to vitamin A. These processes may also depend on the cis isomer configuration as cis-to-trans isomerisation and absorption efficiencies have been shown to differ between 13-cis and 9-cis[38,45–47]. For example, the predominant tissue β-carotene isomer in gerbils administered 13-cis-β-carotene was all-trans-β-carotene, but in gerbils administered 9-cis-β-carotene, the major isomer was 9-cis-β-carotene[45].

The lack of effect of the cis-to-trans ratio on bioconversion factors observed in gerbils fed similar amounts of β-carotene from cassava and carrot is inconsistent with the idea that the trans isomer has a substantially better vitamin A value. Small incremental feeding of cassava may allow for more efficient absorption and bioconversion of cis isomers. The daily doses administered in prior studies[45] were >four times higher than the β-carotene consumed from the cassava and carrot[43] feeds. Other factors such as vitamin A status, feeding mechanism and experimental duration also contribute to potential differences between studies. Due to the large cis content of raw (approximately 20–25 %) and processed (approximately 30–50 %) cassava, it is important to understand the contribution of cis-β-carotene to vitamin A pools when consumed as a staple food. If cis isomers are not as effective as trans, then strategies to improve provitamin A food sources may require targeting of the trans isomer during breeding and/or development of food preparation methods that minimise cis-β-carotene production and maximise all-trans-bioavailability.

In Expt 2, the gerbil model maintained relatively constant liver stores of vitamin A in response to increasing carotenoid intake, and hepatic β-carotene increased. This moderating at adequate liver stores prevents hypervitaminosis A from provitamin A food sources[48]. In studies investigating the

bioaccessibility of carotenoids from cassava, partitioning of β-carotene into micelles during digestion and accumulation of *trans*-β-carotene was linearly proportional to *trans*-β-carotene in cassava[38]. Furthermore, the accumulation of *trans*-β-carotene by human Caco-2 cells was proportional to the concentration in the micelles indicating that bioaccessibility is directly related to *trans*-β-carotene in cassava[38]. Because the gerbils in the current study were not deficient, bioconversion of β-carotene to vitamin A was reduced, more β-carotene was stored, and hepatic vitamin A did not differ with respect to the variable carotenoid content.

The depletion phase was not intended to initiate vitamin A deficiency, but designed to deplete vitamin A reserves. The white cassava feed contained 0·16 nmol β-carotene/g and theoretically provided 3 nmol vitamin A/d or a total of 84 nmol vitamin A for the 4-week depletion period. Comparing with baseline white maize groups from other similar studies[31,39,43], liver reserves were 7–54 % greater in gerbils on the white cassava feed. Thus, the provitamin A content in the white cassava illustrates the contribution of consuming small, regular amounts of provitamin A to vitamin A status. More efficient bioconversion of provitamin A to vitamin A and/or to increased conservation of vitamin A occurs when vitamin A is limited. This phenomenon has been reported in human studies[34,49], but is not incorporated into estimates of dietary conversion factors[4].

The present study confirms that provitamin A carotenoids in cassava are as bioavailable as β-carotene supplements in a vitamin A-depleted gerbil model. Furthermore, results indicate that *cis*-β-carotene may be more efficacious than previously thought. Further studies are needed to fully determine the role of *cis*-β-carotene in maintaining vitamin A status and how it will affect breeding efforts. Bioconversion factors for cassava, high-lycopene carrots and maize in vitamin A-depleted gerbils are much lower than values proposed by the Institute of Medicine[4] and are more likely representative of depleted or deficient human subjects. The present study demonstrates the potential for positively altering or maintaining vitamin A status using cassava with enhanced provitamin A carotenoids and indicates that evaluation in man should be pursued.

Acknowledgements

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