

## Mongolian Gerbils Can Utilize Provitamin-A Carotenoids in Deep-Fried Carrot Chips<sup>1</sup>

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**ABSTRACT** Deep-fried carrot chips, containing provitamin-A carotenes, were developed as an alternative mode of dietary intervention to combat vitamin A deficiency. The biological use of carotenoids in this product as vitamin A precursors was evaluated in Mongolian gerbils. Male 4-wk-old gerbils were fed a diet containing all essential nutrients for 1 wk. Then six gerbils were killed, and the remaining gerbils were fed the diet without vitamin A for 6 wk to produce marginal vitamin A deficiency. After depletion, six gerbils were killed and the remainder divided into four groups of 12 gerbils each and fed vitamin A-containing diet (+VA),  $\beta$ -carotene-containing diet (BC), carrot chip-containing diet (CC), or diet containing no vitamin A/provitamin-A carotenes (-VA). The first three diets contained  $\sim 6 \mu\text{g RE/g}$ . Six gerbils from each group were killed after 2 wk of consuming these diets, and 6 after 4 wk. Final body weight and weekly food consumption did not differ among groups after 2 or 4 wk of repletion. Total liver vitamin A stores of BC and CC gerbils killed after 4 wk of repletion were not different from those of gerbils killed before depletion, but those of -VA gerbils were significantly lower ( $P < 0.05$ ) and those of +VA gerbils were significantly higher ( $P < 0.05$ ). Plasma retinol levels of gerbils killed after 4 wk of repletion, including the -VA group, did not differ. Total liver  $\alpha$ - and  $\beta$ -carotenes and 9-cis  $\beta$ -carotene contents of the CC group were significantly higher ( $P < 0.05$ ) than in the BC group after 4 wk of repletion. This carrot chip product effectively reversed vitamin A deficiency in gerbils. J. Nutr. 132: 211–217, 2002.

**KEY WORDS:** • carrot chips • carotenoids • vitamin A • gerbil

Vitamin A is important for vision, gene expression, reproduction, embryonic development, growth, and immune function (1). Humans need  $< 1 \text{ mg}$  of vitamin A daily to maintain health, yet it was estimated that 3 to 10 million children, mostly in developing countries, become xerophthalmic and 250,000 to 500,000 go blind annually (2). An additional 250 million children under 5 y of age were estimated to be subclinically vitamin A-deficient and at risk of severe morbidity and premature death (3).

Due to the enormous cost of vitamin A deficiency to society, vitamin A intervention is of importance. Vitamin A intervention approaches are commonly grouped into two main control strategies: direct increase in vitamin A intake through dietary modification with natural or fortified foods and supplements and indirect public health measures to control disease frequency (4). For improving vitamin A status, food-based approaches could be most effective where there is widespread availability, variability, adequacy, and acceptability of vitamin A-containing foods among targeted populations (3). In addition, food-based approaches deserve attention because they are

more likely to be sustainable in the long term and will increase the intake of other nutrients simultaneously (5). Such foods are vegetables and fruits that have high provitamin-A carotenoid concentrations, especially  $\beta$ -carotene.

Deep-fried carrot chips that are high in provitamin-A carotenoids were developed in our laboratory as an alternative product for intervention programs to overcome vitamin A deficiency (6). Carrot chips contain fat, which is important and a key factor in carotene absorption (7,8). Underwood (4) reported that vegetable food-based interventions in vitamin A-deficient areas can successfully improve vitamin A status, particularly when dietary fat levels are also increased sufficiently. Carrot chips have a pleasant taste and an appealing appearance; laboratory sensory evaluation and consumer studies indicated that this product was acceptable to both American and Southeast Asian consumers (unpublished data). The retention of provitamin-A carotenoids during storage was above 80%, which is important for optimal usage of this product (9).

Using the conversion factors given by the National Research Council in 1989 (10), the deep-fried carrot chips developed in our laboratory had vitamin A activities of 7322–8532  $\mu\text{g}$  retinol equivalents (RE)<sup>4</sup>/100 g chips (11). Using the new conversion factors (1), this product had vitamin A activities of 3661–4266  $\mu\text{g}$  retinol activity equivalents (RAE) per 100 g chips, an amount high enough to satisfy the human adult daily need for vitamin A. However, to accurately measure the biological activity of provitamin-A carotenoids in the carrot

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<sup>4</sup> Abbreviations used: BC,  $\beta$ -carotene-containing diet; CC, carrot chip-containing diet; RAE, retinol activity equivalents; RE, retinol equivalents; +VA, vitamin A-containing diet; -VA, diet containing no vitamin A/provitamin-A carotenes.

chips, a biological activity study using animal models was conducted.

The appropriate animal model should closely mimic the human uptake, absorption, and metabolism of vitamin A,  $\beta$ -carotene, and other carotenoids (12). Recently, the Mongolian gerbil (*Meriones unguiculatus*) has been suggested as a possible model for carotenoid metabolism studies (13). Mongolian gerbils can both cleave  $\beta$ -carotene and absorb it intact (14). House et al. (13) demonstrated that male gerbils grow normally when fed the AIN-93G rodent diet and absorb large amounts of  $\beta$ -carotene. Lee et al. (15) indicated that gerbils convert  $\beta$ -carotene to vitamin A at a ratio similar to that of humans. Therefore, Lee et al. (15) suggested that the Mongolian gerbil is an appropriate animal model for evaluation of the conversion of  $\beta$ -carotene to vitamin A.

This study was designed to evaluate the biological utilization of carotenoids present in the newly developed deep-fried carrot chip product and to examine whether this chip product could be used to improve the vitamin A status of the Mongolian gerbil.

## MATERIALS AND METHODS

**Deep-fried carrot chips.** Deep-fried carrot chips were developed in our laboratory based on the treatment that resulted in optimal carotenoid retention and the best sensory acceptability (6,9,11). Carrot chips were packaged in vacuum-sealed layered film (2.50 mil, or 0.0625-mm thick, metallized polyester and linear low density polyethylene) pouches (16.5 cm  $\times$  20.3 cm o.d.; Kapak, Minneapolis, MN) using a Multivac AG 500/AG900 (Multivac Kansas City, MO) and stored at  $-50^{\circ}\text{C}$  until ready for use in the diet. The carrot chips

were ground and analyzed before use in the diet. The mean composition of the deep-fried carrot chips was as follows: (per 100 g) energy, 2604.3 kJ (622 kcal); moisture, 3.3 g; crude fat, 56.8 g; crude protein, 3.8 g; dietary fiber, 9.8 g; ash, 2.3 g; and vitamin A activity, 4050 RAE.

**Animals.** After approval by the University's Laboratory Animal Care and Use Committee, male 4-wk-old (weanling) Mongolian gerbils ( $n = 72$ ) with average weights of 28–32 g were obtained from Harlan Sprague-Dawley (Madison, WI). Upon arrival, the gerbils were individually housed in suspended cages. The gerbils had free access to food and distilled water, and room lighting was on a diurnal cycle (12-h light:12-h dark).

**Diets.** The powdered diet described by Thatcher et al. (16), purchased from Harlan Teklad (Madison, WI), was used (Table 1). The composition of this diet is consistent with the recommendations of the National Research Council (17) except for vitamin A. The above diet contained vitamin A (20.9 nmol/g diet as retinyl palmitate in beadlet form) (15). This diet was modified to contain either no vitamin A (including no provitamin-A carotenoids),  $\beta$ -carotene (65.1 nmol/g diet in beadlet form) (15), or ground carrot chips (amount of carrot chips equivalent to an estimated vitamin A activity of 20.9 nmol/g diet). All diets, except the diet lacking vitamin A, had equivalent estimated vitamin A activities (6  $\mu\text{g}$  RE/g diet) using the conversion factors given by the National Research Council (10). These four diets were adjusted to be equal in fat content. The diets were stored at  $5^{\circ}\text{C}$  until used. Before feeding and after several months of storage, the diets were analyzed for retinoid and carotenoid content (18).

**Experimental design.** The gerbils were fed the powdered diet containing vitamin A (20.9 nmol/g diet) for 1 wk. After acclimation for 1 wk, six randomly selected gerbils were killed and the remainder fed the diet without vitamin A for 4 or for 6 wk to produce marginal vitamin A status. After this depletion period, six randomly selected

TABLE 1

Composition of powdered diet fed to gerbils during adaptation, depletion, and repletion periods<sup>1</sup>

Ingredient	+VA diet <sup>2</sup>	–VA diet	BC diet	CC diet
	g/kg			
Casein	200	200	200	200
<i>d</i> /l-Methionine	3	3	3	3
Dextrose, monohydrate	392.7899	392.8299	392.4699	367.8247
Sucrose	200	200	200	200
Safflower oil	58	58	58	58
Partially hydrogenated soybean oil	42	42	42	—
Cellulose	50	50	50	43
Mineral mix (Hegstead IV) <sup>3</sup>	50	50	50	50
Biotin	0.0004	0.0004	0.0004	0.0004
Vitamin B-12 (0.1% in mannitol)	0.03	0.03	0.03	0.03
Calcium pantothenate	0.066	0.066	0.066	0.066
Choline dihydrogen citrate	3.5	3.5	3.5	3.5
Folic acid	0.002	0.002	0.002	0.002
Inositol	0.11	0.11	0.11	0.11
Menadione	0.05	0.05	0.05	0.05
Niacin	0.099	0.099	0.099	0.099
Pyridoxine hydrochloride	0.022	0.022	0.022	0.022
Riboflavin	0.022	0.022	0.022	0.022
Thiamin hydrochloride	0.022	0.022	0.022	0.022
Vitamin D-3, cholecalciferol (500,000 IU/g)	0.0044	0.0044	0.0044	0.0044
<i>d</i> /l- $\alpha$ -tocopherul acetate (500 IU/g)	0.2423	0.2423	0.2423	0.2423
Vitamin A palmitate (500,000 IU/g)	0.04	—	—	—
$\beta$ -carotene, beadlet form (10%)	—	—	0.36	—
Ground carrot chips	—	—	—	74

<sup>1</sup> Described by Thatcher et al. (17).

<sup>2</sup> +VA diet contained vitamin A; –VA diet contained no vitamin A or carotenoids; BC diet contained  $\beta$ -carotene, and CC diet contained carrot chips.

<sup>3</sup> The mineral mix (Hegstead IV, Teklad, Madison, WI) supplied the following (g/kg diet): calcium carbonate, 299.7452; potassium phosphate, dibasic, 322.226; calcium phosphate, dibasic, 74.9363; magnesium sulfate, 101.9134; sodium chloride, 167.3577; ferric citrate, USP (16.7% Fe), 27.4766; potassium iodide, 0.79932; manganese sulfate, 3.78527; zinc chloride, 0.249788; cupric sulfate, 0.299745; and cellulose, 1.210677.

gerbils were killed, and the remaining gerbils were randomly divided into four groups of 12 gerbils each and fed either: vitamin A-containing diet (+VA group),  $\beta$ -carotene-containing diet (BC group), carrot chip-containing diet (CC group); these three diets contained similar vitamin A activity  $\sim 6 \mu\text{g RE/g}$  diet), or a diet containing no vitamin A or provitamin-A carotenoids ( $-VA$  group). Six gerbils from each of the groups were killed after 2 wk of consuming these diets, and the other six after 4 wk. This experimental design is summarized in Figure 1. All gerbils were weighed and food consumption determined weekly.

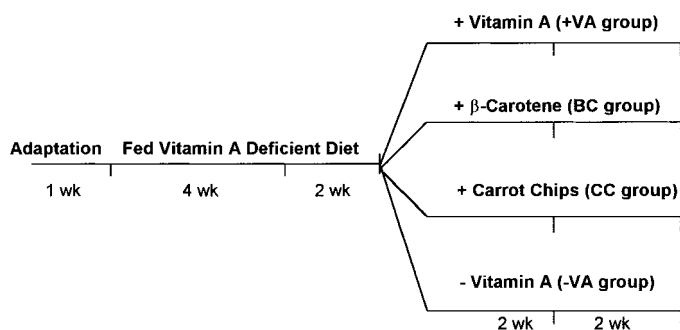
**Tissue sampling and analyses.** At the time of killing, blood samples were collected using heparinized syringes via cardiac puncture in gerbils anesthetized with a mixture of ketamine hydrochloride (Vetlar; Parke-Davis, Morris Plains, NJ) and xylazine (Rompun; Miles Laboratory, Shawnee, KS; 90:10, v/v), delivered by intramuscular injection (0.1 mL/100 g body), as recommended by Lee et al. (15). Blood samples were kept in ice, centrifuged at  $3000 \times g$  at  $5^\circ\text{C}$  for 10 min, and plasma frozen at  $-50^\circ\text{C}$  for future analyses. Livers were removed, weighed, placed immediately on dry ice, and stored for future analyses at  $-50^\circ\text{C}$ .

The method of Nierenberg and Nann (19) was utilized for plasma carotenoid and retinoid analyses. The liver was extracted according to Lederman et al. (12) with the following modification: the residue obtained following hexane extraction and evaporation was resuspended with 100  $\mu\text{L}$  tetrahydrofuran, then 50  $\mu\text{L}$  mobile phase was added, and the mixture was sonicated. The volume was brought to 500  $\mu\text{L}$  by adding 350  $\mu\text{L}$  mobile phase, and 20  $\mu\text{L}$  was injected into the HPLC system for carotenoid (450 nm) and retinoid (325 nm) analyses.

The HPLC system consisted of the following Waters Associates (Milford, MA) equipment: 600E solvent delivery system, Pheodyne, 484 UV detector, and 74SB integrator. The separation was carried out using a reversed phase Microsorb-MV (5- $\mu\text{m}$ ,  $250 \times 4.6$  mm)  $\text{C}_{18}$  column (Rainin, Woburn, MA) that was protected with a guard column of  $\text{C}_{18}$  material (3 cm length  $\times$  4.6 mm i.d.) packed with spheri-5- $\text{C}_{18}$  (5- $\mu\text{m}$  particle size). The mixture of acetonitrile:tetrahydrofuran:methanol:1% ammonium acetate (65:25:6:4) was used as the mobile phase under isocratic conditions (19,20). Minimum detectable levels (per injection) for  $\alpha$ -carotene,  $\beta$ -carotene, and retinol were 0.075, 0.093, and 0.175 pmol, respectively. The percentage of recoveries of spiked standards of carotenes and retinol were between 92 and 101, and the CV were  $<6\%$ .

Plasma triglyceride, total cholesterol, and HDL cholesterol were analyzed according to Carr et al. (21). LDL + VLDL cholesterol was calculated by subtracting the HDL cholesterol from total cholesterol.

**Statistical analyses.** Data were analyzed by using the General Linear Model procedure of SAS, Version 6 (SAS Institute Inc., Cary, NC). Duncan's multiple range tests were performed for multiple comparisons to determine differences among groups. Differences were considered significant at  $P < 0.05$ . Values were expressed as group means  $\pm$  SD.



Six gerbils were killed at each cross-bar marking.

**FIGURE 1** Experimental design of gerbil-feeding trial with  $\beta$ -carotene-containing (BC), carrot chip-containing (CC), vitamin A-containing (+VA), and containing no vitamin A/provitamin-A carotenoids ( $-VA$ ) diets.

**TABLE 2**

*Final body weight and food consumption of gerbils at baseline, after depletion, and after repletion with different diets (BC, CC, +VA, and  $-VA$  diets) for 2 or 4 wk<sup>1,2</sup>*

Experimental diet	Final body weight	Final weekly food consumption
	g	
Baseline	42.3 $\pm$ 2.4 <sup>c</sup>	38.2 $\pm$ 1.7
Depleted 4 wk	51.8 $\pm$ 5.7 <sup>b</sup>	42.3 $\pm$ 3.0
Depleted 6 wk	56.4 $\pm$ 4.5 <sup>b</sup>	38.4 $\pm$ 4.6
BC repleted 2 wk	60.7 $\pm$ 4.9 <sup>ab</sup>	37.7 $\pm$ 4.8
BC repleted 4 wk	60.5 $\pm$ 7.6 <sup>ab</sup>	40.8 $\pm$ 2.5
CC repleted 2 wk	62.1 $\pm$ 7.5 <sup>ab</sup>	38.0 $\pm$ 5.0
CC repleted 4 wk	60.5 $\pm$ 5.0 <sup>ab</sup>	36.1 $\pm$ 4.0
+VA repleted 2 wk	60.1 $\pm$ 3.7 <sup>ab</sup>	38.0 $\pm$ 2.8
+VA repleted 4 wk	64.7 $\pm$ 4.8 <sup>a</sup>	40.0 $\pm$ 2.8
$-VA$ 2 wk	62.8 $\pm$ 3.9 <sup>ab</sup>	37.2 $\pm$ 3.3
$-VA$ 4 wk	61.2 $\pm$ 4.3 <sup>ab</sup>	40.0 $\pm$ 3.8

<sup>1</sup> Values are means  $\pm$  SD,  $n = 6$ . Values within a column with the same superscripts are not different,  $P \geq 0.05$ .

<sup>2</sup> Gerbils on baseline fed vitamin A-containing diet; gerbils on depletion fed vitamin A-deficient diet; gerbils on repletion fed either BC,  $\beta$ -carotene-containing diet; CC, carrot chip-containing diet; or +VA, vitamin A-containing diet. These three diets had vitamin A activity  $\sim 6 \mu\text{g RE/g}$  diet. The  $-VA$  groups continued on depleted diet for an additional 2 and 4 wk period.

## RESULTS

**Food consumption and growth performance.** The growth performance and weekly diet consumption of gerbils during adaptation, depletion, and repletion periods are presented in Table 2. There were no significant effects ( $P \geq 0.05$ ) of diet on weekly food intakes and body weights of the gerbils. There were no significant differences ( $P \geq 0.05$ ) in final body weight and weekly food consumption among the four groups.

**Liver stores of vitamin A.** The gerbils were marginally deficient in vitamin A (15) after consuming the vitamin A-deficient diet for 6 wk as the liver retinol decreased from 0.51  $\mu\text{mol/g}$  (0.94  $\mu\text{mol/liver}$ ) to 0.20  $\mu\text{mol/g}$  (0.60  $\mu\text{mol/liver}$ ; Table 3). Liver vitamin A stores of the BC and CC groups returned to predepletion (or baseline) levels after 4 wk of repletion, and after 2 wk of repletion for +VA gerbils. After being repleted for 4 wk, the liver vitamin A stores were not significantly different from the baseline for BC and CC groups, were higher for the +VA groups, and were lower in the  $-VA$  group ( $P < 0.05$ ).

**Plasma retinol.** The mean plasma retinol concentrations of the BC, CC, +VA, and  $-VA$  groups after 4 wk of repletion were: 1.47, 1.33, 1.40, and 1.40  $\mu\text{mol/L}$  (42.17, 38.06, 40.16, and 40.12  $\mu\text{g/dL}$ ), respectively (Table 3), which, except for the BC group, were not different ( $P \geq 0.05$ ) from baseline. Plasma retinol did not differ among gerbils in the four diet groups after 4 wk repletion. Serum levels  $< 0.7 \mu\text{mol/L}$  (20  $\mu\text{g/dL}$ ) are considered to be indicative of subclinical vitamin A inadequacy (4) or marginal deficiency in humans. There was no significant correlation between plasma retinol concentrations and liver stores of retinol.

**Plasma and liver carotenoids.**  $\alpha$ -Carotene,  $\beta$ -carotene, and 9-*cis*  $\beta$ -carotene were not detectable in plasma of gerbils in all diet groups nor were  $\alpha$ -carotene,  $\beta$ -carotene, and 9-*cis*  $\beta$ -carotene in livers of gerbils fed the +VA and  $-VA$  diets. Livers



TABLE 3

*Liver retinol stores and plasma retinol concentrations of gerbils at baseline, after depletion, and after repletion with different diets (BC, CC, +VA, and -VA diets) for 2 or 4 wk<sup>1,2</sup>*

Experimental diet	Liver retinol stores $\mu\text{mol/liver}$	Plasma retinol $\mu\text{mol/L}$
Baseline	0.94 $\pm$ 0.07 <sup>bc</sup>	1.18 $\pm$ 0.07 <sup>c</sup>
Depleted 4 wk	0.91 $\pm$ 0.17 <sup>bc</sup>	1.46 $\pm$ 0.21 <sup>ab</sup>
Depleted 6 wk	0.60 $\pm$ 0.11 <sup>dc</sup>	1.45 $\pm$ 0.09 <sup>ab</sup>
BC repleted 2 wk	0.65 $\pm$ 0.10 <sup>de</sup>	1.57 $\pm$ 0.28 <sup>a</sup>
BC repleted 4 wk	0.90 $\pm$ 0.26 <sup>bc</sup>	1.47 $\pm$ 0.23 <sup>ab</sup>
CC repleted 2 wk	0.77 $\pm$ 0.13 <sup>cd</sup>	1.31 $\pm$ 0.17 <sup>bc</sup>
CC repleted 4 wk	0.95 $\pm$ 0.25 <sup>bc</sup>	1.33 $\pm$ 0.08 <sup>bc</sup>
+VA repleted 2 wk	1.04 $\pm$ 0.18 <sup>b</sup>	1.32 $\pm$ 0.09 <sup>bc</sup>
+VA repleted 4 wk	1.64 $\pm$ 0.31 <sup>a</sup>	1.40 $\pm$ 0.14 <sup>abc</sup>
-VA 2 wk	0.53 $\pm$ 0.08 <sup>e</sup>	1.42 $\pm$ 0.09 <sup>ab</sup>
-VA 4 wk	0.44 $\pm$ 0.14 <sup>e</sup>	1.40 $\pm$ 0.24 <sup>abc</sup>

<sup>1</sup> Values are means  $\pm$  SD,  $n = 6$ . Values within a column with the same superscripts are not different,  $P \geq 0.05$ . nd = not detectable.

<sup>2</sup> Gerbils on baseline fed vitamin A-containing diet; gerbils on depletion fed vitamin A-deficient diet; gerbils on repletion fed either BC,  $\beta$ -carotene-containing diet; CC, carrot chip-containing diet; or +VA, vitamin A-containing diet. These three diets had vitamin A activity  $\sim 6 \mu\text{g RE/g}$  diet. The -VA groups continued on depleted diet for an additional 2 or 4 wk period.

of gerbils fed the CC diet contained  $\alpha$ - and  $\beta$ -carotenes and 9-cis  $\beta$ -carotene, and those fed the BC diet contained only  $\beta$ -carotene and 9-cis  $\beta$ -carotene (Table 4). Liver  $\beta$ -carotene and 9-cis  $\beta$ -carotene stores of the CC group were significantly higher ( $P < 0.05$ ) than those of the BC group after 4 wk of repletion, but not after 2 wk (Table 4).

**Plasma triglyceride and cholesterol.** Plasma triglyceride and HDL cholesterol concentrations did not differ among groups after 2 wk repletion (Table 5). However, plasma LDL + VLDL cholesterol and total cholesterol concentrations of BC-fed gerbils after 2 wk were significantly different ( $P < 0.05$ ) from those fed +VA. Plasma triglyceride, LDL + VLDL cholesterol, HDL cholesterol, and total cholesterol concentrations did not differ among groups after 4 wk of repletion. No significant correlations were observed between carotene concentrations and all the plasma lipid concentrations.

## DISCUSSION

In the current study, growth performance was not shown to be a good indicator for evaluation of carotenoid bioavailability and bioconversion. Similar findings had previously been reported by other researchers (15,16,22,23). In our study, the gerbils were only depleted until reaching marginal deficiency, and no visible signs of deficiency were observed in these depleted gerbils.

Vitamin A content of the liver is the most commonly used variable for assessing the vitamin A potency of natural provitamin-A carotenoids (24). Olson (25) reported that in healthy individuals,  $\sim 90\%$  of vitamin A in the body is stored in the liver, and this percentage decreases to 50% or less in severely deficient individuals. Hepatic vitamin A stores, thus, can be interpreted to reflect nutrient adequacy to meet total body needs, barring factors that impede their release into circulation (1). Liver retinol stores may originate from preformed dietary retinoids or from provitamin-A carotenes by enzymatic con-

version (24). Hepatic retinol stores of the gerbils in the CC group after 4 wk of repletion was more than double that of the nonrepleted group (-VA). Provitamin-A carotenoids present in deep-fried carrot chips were biologically available in that vitamin A suboptimacy was reversed by supplementation with the carrot chip diet containing similar amounts of carotenes as the  $\beta$ -carotene-containing diet. The liver vitamin A stores in the CC group were significantly higher than in the nonrepleted group (-VA), indicating that carotenes in the carrot chips were absorbed and then converted into vitamin A. Lee et al. (15), using diets containing the same amounts of vitamin A or  $\beta$ -carotene as in the current study, found that liver vitamin A levels in gerbils were reversed after 31 d of repletion.

Based on the provitamin-A carotenoid analyses using HPLC and vitamin A activity calculations using the RAE (1), a serving of 30 g deep-fried carrot chip product contained enough vitamin A activity to satisfy the vitamin A requirement of human adults. In this research, the investigators examined whether the provitamin-A carotenoids of the carrot chips were biologically available and able to reverse vitamin A deficiency by monitoring the growth performance and retinoid and carotenoid levels in liver and plasma of the Mongolian gerbils. The CC, BC, and +VA diets had equivalent estimated vitamin A activities as RE, based on the conversion factors given by the National Research Council (10). However, the liver stores of retinol of CC and BC groups after 4 wk of repletion were only 55–58% of that of the +VA group. Hence, the efficiency in which the provitamin-A carotenoids were converted into retinol in the liver is in agreement with the new conversion factors using RAE (1), where 1  $\mu\text{g}$  RAE is equal to 12  $\mu\text{g}$   $\beta$ -carotene or 24  $\mu\text{g}$   $\alpha$ -carotene.

Plasma retinol concentration was not a good indicator of vitamin A deficiency in this study. Lee et al. (15) reported that serum retinol concentrations of gerbils did not differ among several dietary groups, including a group fed no vitamin A or

TABLE 4

*Liver stores of carotenes of gerbils at baseline, after depletion, and after repletion with different diets (BC, CC, +VA, and -VA diets) for 2 or 4 wk<sup>1,2</sup>*

Experimental diet	$\alpha$ -carotene	$\beta$ -carotene	9-cis $\beta$ -carotene
$\text{nmol/liver}$			
Baseline	nd	nd	nd
Depleted 4 wk	nd	nd	nd
Depleted 6 wk	nd	nd	nd
BC repleted 2 wk	nd	8.47 $\pm$ 3.15 <sup>b</sup>	2.33 $\pm$ 0.61 <sup>b</sup>
BC repleted 4 wk	nd	14.73 $\pm$ 4.62 <sup>b</sup>	3.82 $\pm$ 0.50 <sup>b</sup>
CC repleted 2 wk	9.87 $\pm$ 2.50 <sup>b</sup>	12.55 $\pm$ 3.17 <sup>b</sup>	2.81 $\pm$ 0.80 <sup>b</sup>
CC repleted 4 wk	16.50 $\pm$ 6.59 <sup>a</sup>	22.39 $\pm$ 9.68 <sup>a</sup>	5.48 $\pm$ 2.16 <sup>a</sup>
+VA repleted 2 wk	nd	nd	nd
+VA repleted 4 wk	nd	nd	nd
-VA 2 wk	nd	nd	nd
-VA 4 wk	nd	nd	nd

<sup>1</sup> Values are means  $\pm$  SD,  $n = 6$ . Values within a column with the same superscripts are not different,  $P \geq 0.05$ . nd = not detectable.

<sup>2</sup> Gerbils on baseline fed vitamin A-containing diet; gerbils on depletion fed vitamin A-deficient diet; gerbils on repletion fed either BC,  $\beta$ -carotene-containing diet; CC, carrot chip-containing diet; or +VA, vitamin A-containing diet. These three diets had vitamin A activity  $\sim 6 \mu\text{g RE/g}$  diet. The -VA groups continued on depleted diet for an additional 2 and 4 wk period.

TABLE 5

Plasma triglyceride, LDL+VLDL-, HDL-, and total cholesterol concentrations of gerbils at baseline, after depletion, and after repletion with different diets (BC, CC, +VA, and -VA diets) for 2 or 4 wk<sup>1,2</sup>

Experimental diet	Triglyceride	LDL+VLDL-cholesterol	HDL-cholesterol	Total cholesterol
	mmol/L			
Baseline	0.073 ± 0.032 <sup>c</sup>	1.64 ± 0.33 <sup>abc</sup>	0.83 ± 0.06 <sup>c</sup>	2.47 ± 0.34 <sup>c</sup>
Depleted 4 wk	0.126 ± 0.061 <sup>bc</sup>	1.55 ± 0.51 <sup>abc</sup>	1.54 ± 0.48 <sup>b</sup>	3.08 ± 0.98 <sup>abc</sup>
Depleted 6 wk	0.172 ± 0.081 <sup>abc</sup>	1.67 ± 0.53 <sup>ab</sup>	1.80 ± 0.35 <sup>ab</sup>	3.47 ± 0.78 <sup>ab</sup>
BC repleted 2 wk	0.272 ± 0.146 <sup>ab</sup>	1.74 ± 0.33 <sup>a</sup>	2.09 ± 0.27 <sup>a</sup>	3.83 ± 0.45 <sup>a</sup>
BC repleted 4 wk	0.187 ± 0.055 <sup>abc</sup>	0.95 ± 0.33 <sup>d</sup>	1.93 ± 0.27 <sup>ab</sup>	2.88 ± 0.42 <sup>bc</sup>
CC repleted 2 wk	0.274 ± 0.104 <sup>ab</sup>	1.22 ± 0.48 <sup>abcd</sup>	2.02 ± 0.35 <sup>a</sup>	3.24 ± 0.47 <sup>abc</sup>
CC repleted 4 wk	0.292 ± 0.207 <sup>ab</sup>	0.93 ± 0.50 <sup>d</sup>	2.14 ± 0.47 <sup>a</sup>	3.07 ± 0.89 <sup>abc</sup>
+VA repleted 2 wk	0.239 ± 0.086 <sup>abc</sup>	1.09 ± 0.34 <sup>cd</sup>	1.88 ± 0.21 <sup>ab</sup>	2.97 ± 0.28 <sup>bc</sup>
+VA repleted 4 wk	0.313 ± 0.213 <sup>a</sup>	0.73 ± 0.22 <sup>d</sup>	2.11 ± 0.31 <sup>a</sup>	2.84 ± 0.33 <sup>bc</sup>
-VA 2 wk	0.245 ± 0.086 <sup>abc</sup>	1.18 ± 0.37 <sup>bcd</sup>	1.90 ± 0.28 <sup>ab</sup>	3.08 ± 0.63 <sup>abc</sup>
-VA 4 wk	0.177 ± 0.091 <sup>abc</sup>	0.73 ± 0.27 <sup>d</sup>	1.91 ± 0.19 <sup>ab</sup>	2.64 ± 0.25 <sup>bc</sup>

<sup>1</sup> Values are means ± SD, *n* = 6. Values within a column with the same superscripts are not different, *P* ≥ 0.05.

<sup>2</sup> Gerbils on baseline fed vitamin A-containing diet; gerbils on depletion fed vitamin A-deficient diet; gerbils on repletion fed either BC, β-carotene-containing diet; CC, carrot chip-containing diet; or +VA, vitamin A-containing diet. These three diets had vitamin A activity ~6 μg RE/g diet. The -VA groups continued on depleted diet for an additional 2 and 4 wk period.

β-carotene. The Institute of Medicine (1) indicated that only when liver vitamin A reserves fall below a critical concentration, thought to be ~20 μg/g liver (0.070 μmol/g), will plasma retinol levels decrease. Because the liver stores of retinol of all gerbils in the current study were >58 μg/g liver (0.203 μmol/g), the plasma retinol concentration was unchanged. Because of the relatively insensitive relationship between plasma retinol concentration and liver vitamin A in the adequate range of intake and because of the potential for confounding factors to influence the levels and interpretation of plasma retinol concentrations, plasma retinol concentration was not chosen as a primary status indicator for human populations for estimating an average requirement for vitamin A (1).

Van het Hof et al. (26) reported that the bioavailability of β-carotene from vegetables in particular has been shown to be low (14% from mixed vegetables) compared with that of purified β-carotene added to a sample matrix. Rock et al. (27) reported that bioavailability of β-carotene in processed carrots was higher than in raw carrots. By measuring serum response of healthy men to a single ingestion, Huang et al. (28) found that the bioavailability of stir-fried carrots was 33% that of β-carotene beadlets. In this study, the liver vitamin A stores of the CC group after 4 wk of repletion was not significantly different from the BC group. This finding indicated that the provitamin-A carotenoids in the carrot chips were highly bioavailable. Deep-frying may also increase their bioavailability. In the current study, the CC diet successfully reversed the marginal vitamin A deficiency in gerbils similarly to those fed the BC diet. The consumption of deep-fried carrot chips potentially can reverse marginal vitamin deficiency in humans.

In this study, we did not detect any α-carotene, β-carotene, or 9-cis β-carotene in the plasma of gerbils fed with BC and CC diets. Perhaps the amounts of these carotenoids available in BC and CC diets were too low and all were transferred to the liver or converted into retinoids. Using the same amount of β-carotene (3 μg RAE/g diet or 67.1 nmol/g diet) as used in this study, Lee et al. (15) likewise did not detect β-carotene in the plasma of gerbils. Only when these researchers used a doubled amount of β-carotene (145.9 nmol/g diet) did they detect the β-carotene in the plasma of gerbils fed the BC diet. A feeding trial was performed by Tee et al. (22) on rats. The

rats were fed commercial rat pellets containing 2600 μg RAE/kg diet, and the rats were supplemented with either retinol concentrate (30–45 μg RAE in 0.2 mL corn oil), β-carotene concentrate (30–45 μg RAE in 0.2 mL corn oil, freeze-dried carrots (30 μg RAE), freeze-dried swamp cabbage (30 μg RAE), or control (0.2 mL corn oil). As in the study by Lee et al. (15), β-carotene was not detected in the serum of rats in all groups (22). Perhaps the carotenoids were cleared from the plasma or converted into vitamin A quickly as a response to the body's need for retinol.

In this study 9-cis β-carotene was detected in the livers of gerbils in the BC and CC groups. It was expected that 9-cis β-carotene would be found in the livers of the CC gerbils because the carrot chips contained a large amount of 9-cis β-carotene, but not in the livers of the BC group. Because 9-cis β-carotene was observed in the livers of gerbils fed the CC diet, this indicates that this isomer was absorbed and biologically available. Levin and Mokady (29) reported that 9-cis β-carotene was preferentially accumulated and acted as a precursor of retinol in chicks. This may be why the CC groups tended to have greater (*P* = 0.3065) liver retinol stores than the BC groups. In the diet formulation and data calculations, the 9-cis β-carotene was not included in the calculation of vitamin A activity. Sweeney and Marsh (30) reported that *cis*-isomers of provitamin-A carotenoids, such as α-carotene, β-carotene, and β-cryptoxanthin, have provitamin-A activities that are ~50% or less of that of corresponding all-trans carotenoids.

The presence of 9-cis β-carotene in the livers of gerbils in the BC group as well as in the CC group may be due to isomerization of the all-trans isomer to 9-cis before or during absorption, or within tissues as hypothesized by Erdman et al. (31) in a ferret study. Deming et al. (32) also observed the isomerization of β-carotene in tissues of gerbils after a dose of the all-trans and 9-cis β-carotene. In contrast to what happened in gerbils and ferrets, there may be an isomerization from *cis* to *trans* isomers in humans. In a study by Johnson et al. (33), 15 men were given oral doses of 9-cis β-carotene and the serum concentrations of 9-cis β-carotene did not increase, perhaps indicating poor absorption, isomerization from the *cis*-to-*trans*- form, or a very rapid tissue uptake. You et al. (34)

demonstrated *cis-trans* isomerization during absorption in humans and reported that a large proportion of the 9-*cis*  $\beta$ -carotene dose was isomerized to *trans*  $\beta$ -carotene before entering the bloodstream and increased the vitamin A value of 9-*cis*  $\beta$ -carotene. Furthermore, Rock et al. (27) found that the daily consumption of processed carrots and spinach over a 4-wk period produced a plasma  $\beta$ -carotene response that averaged three times that associated with the consumption of the same amount of  $\beta$ -carotene from these vegetables in the raw form ( $P = 0.09$ ), despite the greater proportion of 9-*cis*  $\beta$ -carotene isomers provided by the processed forms. These results suggested that the isomerization of  $\beta$ -carotene produced by heat treatment did not negate the enhanced  $\beta$ -carotene uptake associated with consuming cooked and pureed vegetables vs. raw vegetables. Providing cooked and pureed vegetables rather than raw vegetables would seem to be a better approach to providing bioavailable  $\beta$ -carotene from carotenoid-rich foods, which may have applicability for populations who rely on these foods to meet vitamin A requirements.

Carotenoids are believed to have a variety of different actions that are related to decreased risk of some degenerative diseases (35). Therefore, the effects of having the carrot chip product as a component of the diet of gerbils on their plasma levels of triglycerides and cholesterol (LDL + VLDL, HDL, and total) were also evaluated in this study. However, because the amount of carrot chips, vitamin A, or  $\beta$ -carotene added in the diets in this study was likely just enough to satisfy the vitamin A requirement, no significant correlations between the consumption of these diets and the plasma lipids were observed. This is supported by the fact that no carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene, or 9-*cis*  $\beta$ -carotene) were detected in the gerbil plasma in this study. Chi et al. (36) investigated the effects of dietary fats and antioxidants on blood pressure and plasma lipids in spontaneously hypertensive rats. They found that dietary antioxidants, consisting of tocopherol, ascorbic acid, and  $\beta$ -carotene, did not affect plasma lipid levels. Only the type of oil used in the diet influenced the blood pressure and plasma lipids of these rats. Sun et al. (20) and Sulli et al. (37), however, found that supplementation with a combination of  $\alpha$ -tocopherol (0.5074 g/100 g *dl*- $\alpha$ -tocopherol) and  $\beta$ -carotene (25 mg/kg body administered intravenously twice weekly) significantly decreased plasma total and LDL cholesterol concentrations in hypercholesterolemic rabbits. However, this could be expected because the  $\beta$ -carotene intakes of the rabbits in that experiment were higher on a body weight basis than in the current study.

In conclusion, the provitamin-A carotenoids contained in deep-fried carrot chips were biologically available to the gerbils. After 4 wk, the gerbils marginally deficient in vitamin A were repleted with the provitamin-A carotenoids in the carrot chip diet. Therefore, deep-fried carrot chips potentially may reverse marginal vitamin A deficiency in humans.

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