Original Research

Carotenoid profiles in provitamin A-containing fruits and vegetables affect the bioefficacy in Mongolian gerbils

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Abstract

Fruits and vegetables are rich sources of provitamin A carotenoids. We evaluated the vitamin A (VA) bioefficacy of a whole foods supplement (WFS) and its constituent green vegetables (Study 1) and a variety of fruits with varying ratios of provitamin A carotenoids (Study 2) in VA-depleted Mongolian gerbils ($n = 77$/study). After feeding a VA-deficient diet for 4 and 6 weeks in Studies 1 and 2, respectively, customized diets, equalized for VA, were fed for 4 and 3 weeks, respectively. Both studies utilized negative and VA-positive control groups. In Study 1, liver VA was highest in the VA group (0.82 ± 0.16 μmol/liver, $P < 0.05$), followed by brussels sprouts (0.50 ± 0.15 μmol/liver), Betanat® (β-carotene from *Blakeslea trispora*) (0.50 ± 0.12 μmol/liver) and spinach (0.47 ± 0.09 μmol/liver) groups, which did not differ from baseline. The WFS (0.44 ± 0.06 μmol/liver) and kale (0.43 ± 0.14 μmol/liver) groups had lower liver VA than the baseline group ($P < 0.05$), but did not differ from the brussels sprouts, Betanat® and spinach groups. In Study 2, liver VA was highest in the orange (0.67 ± 0.18 μmol/liver), papaya (0.67 ± 0.15 μmol/liver) and VA (0.66 ± 0.14 μmol/liver) groups, followed by the mango (0.58 ± 0.09 μmol/liver) and tangerine (0.55 ± 0.15 μmol/liver) groups. These groups did not differ from baseline. The banana group (0.47 ± 0.15 μmol/liver) was unable to maintain baseline stores of VA and did not differ from the control (0.46 ± 0.13 μmol/liver). These fruits (except banana), vegetables and the WFS were able to prevent VA deficiency in Mongolian gerbils and could be an effective part of food-based interventions to support VA nutrition in developing countries and worldwide.

Keywords: bioconversion factors, carotenoids, provitamin A, supplements, vitamin A

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Introduction

Vitamin A (VA) is essential for vision, immune function, growth, development and reproduction. It occurs as preformed VA found naturally in animal products and provitamin A carotenoids from plant sources. In developing nations, where 70–90% of VA is obtained from provitamin A, VA deficiency is a major health problem. In these populations, food-based strategies that promote dietary diversification are critical in a sustainable effort to eliminate VA deficiency.[1] Alternatively, in well-nourished populations such as the US and Europe, excess VA intake is a growing concern. Daily consumption of ~3.5 times the VA recommended daily allowance (RDA) is common due to a high intake of preformed VA from multivitamins, fish liver oil, butter and fortified foods such as milk, margarine, breakfast cereals and some snack foods.[2] Reports indicate a widespread and increasing use of dietary supplements,[3,4] and many multivitamin formulations provide more than twice the current RDA as preformed VA.[2,5] Regular use of these supplements may contribute to excessive nutrient intake.[4]

While symptoms of chronic hypervitaminosis A may manifest at daily doses of preformed VA >10 times the RDA after months or years,[6] recent epidemiological studies report that daily consumption of ~2 times the RDA may be associated with reductions in bone mineral density and hip fracture.[7,8] Provitamin A carotenoids, however, do not cause hypervitaminosis A due to regulated conversion to VA,[9] and may be a safer alternative to preformed VA when taken at physiological doses. Unlike supplementation with pharmacological doses of β-carotene that may increase lung cancer incidence in smokers,[10–12] high intakes of β-carotene from food may result in hypercarotenemia, but reports of toxic side-effects are few.[9] Additionally, a recent meta-analysis revealed that intake of pharmacological doses of β-carotene or preformed VA intended as antioxidants increased all-cause mortality,[13] but a study of undernourished women in rural south Asia found that weekly β-carotene doses decreased mortality rates related to pregnancy.[14] Increased fruit and vegetable intakes are consistently associated with decreased incidence of cardiovascular disease.[15,16] The data are mixed for the association between fruit and vegetable intake and cancer,[15] but recent evidence supports whole food sources of VA as more effective than preformed VA for the prevention of breast cancer in rats.[17]

The bioefficacy of provitamin A carotenoids refers to the amount of retinol formed from the amount of provitamin A carotenoids ingested.[18,19] The Institute of Medicine has defined the retinol equivalency ratio of β-carotene from a mixed diet to be 12 μg β-carotene to 1 μg of retinol (12:1) and of other provitamin A carotenoids to be 24:1.[20] These ratios are influenced by factors such as the food matrix and the VA status of the host.[21] Reported equivalency ratios in humans range from 3.8:1 for Golden Rice[22] to 28:1 for vegetables.[23] The VA value of supplements made from whole foods is currently not known. Additionally, some important yellow and orange fruits rich in provitamin A have not been directly compared for their bioefficacy. Two studies were conducted to determine the VA value of a dietary supplement made from whole foods and its major components (i.e. kale, brussels sprouts, spinach and Betanat® [β-carotene from *Blakeslea trispora*]) (Study 1) and a variety of fruits (Study 2) in VA-depleted Mongolian gerbils (*Meriones unguiculatus*). Mongolian gerbils metabolize β-carotene similarly to humans and serve as a model to test the bioefficacy of provitamin A carotenoids,[24] allowing the direct measurement of liver VA.[25]

Materials and methods

Components

In Study 1, the whole foods supplement (WFS) and four of its main components (Betanat®, brussels sprouts [*Brassica oleracea* var. *gemmifera*], kale [*Brassica oleracea* var. *acephala*] and spinach [*Spinacia oleracea*]) were provided by Standard Process Inc (Palmyra, WI, USA). The WFS and vegetables were dried and powdered, and Betanat® was in beadlet form. In Study 2, whole fruits (mango, orange, tangerine and papaya) were purchased from a local grocery store, peeled, chopped, freeze-dried and ground. The banana was in flour form (Makerere University, Uganda) and was used to mimic a staple form of banana consumed in many parts of the world. After acquisition or preparation, all fruit and vegetable components were stored at $-80°$C.

Animals, diets and study design

Male 40-d-old Mongolian gerbils (Studies 1 and 2, $n = 77$/study) were obtained from Charles River Laboratories (Kingston, NY, USA). Gerbils were individually housed in plastic cages, and room temperature and humidity were constant with a 12-h light:dark cycle. Gerbils were accustomed to the dosing procedure with daily 40 μL cottonseed oil doses. Gerbils were weighed daily and monitored for health until all were thriving, at which time, they were weighed every two days. All animal handling procedures were approved by the College of Agriculture and Life Sciences Animal Care and Use Committee of the University of Wisconsin-Madison.

The gerbils were fed a VA- and carotenoid-free, semi-purified, powdered feed (VA-free basal feed) *ad libitum*. In consultation with the staff nutritionist at the feed manufacturer (Harlan-Teklad, Madison, WI, USA), gerbil feeds were individually designed to provide isoenergic and isonitrogenous diets (Table 1). Macronutrient and fiber content (estimated from the USDA Nutrient Database[26]) for each vegetable and fruit diet were equalized by modifying casein, sucrose, cottonseed oil and cellulose. Mixed feeds were stored at $-20°$C. After the depletion phase (Study 1, 4 weeks; Study 2, 6 weeks), a baseline group ($n = 7$) was killed by exsanguination while under isofluorane anesthesia

Table 1 Composition of experimental diets designed in consultation with a nutritionist at the feed supplier (Harlan-Teklad, Madison, WI, USA) that were fed to Mongolian gerbils to determine vitamin A (VA) efficacy of test diets

		Study 1			Study 2				
Ingredient	VA-free (g/kg feed)	Brussels sprout (g/kg feed)	Kale (g/kg feed)	Spinach (g/kg feed)	Banana (g/kg feed)	Mango (g/kg feed)	Orange (g/kg feed)	Tangerine (g/kg feed)	Papaya (g/kg feed)
Casein, vitamin free	200.0	153.9	161.6	184.6	182.9	198.1	184.9	196.8	198.4
L-Cystine	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Sucrose	360.5	355.4	357.5	354.5	60.9	309.4	197.3	318.3	341.2
Maltodextrin	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Corn starch	150.0	79.8	56.8	142.3	150.0	150.0	150.0	150.0	150.0
Cottonseed oil	60.0	56.3	52.7	58.1	55.3	59.1	57.7	58.9	59.7
Cellulose	60.0	13.3	39.1	49.3	22.7	54.2	26.4	53.6	56.0
Mineral mix AIN-93M-MX	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Magnesium oxide	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Ca Phosphate, dibasic	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin mix*	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin E acetate	0.242	0.242	0.242	0.242	0.242	0.242	0.242	0.242	0.242
Vitamin D_3	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Component†	0	171.8	162.7	41.6	358.6	59.4	213.9	52.6	25.0

*Vitamin mix provided the following (mg/kg purified diet): biotin, 0.4; calcium pantothenate, 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
†Vegetables were dried and powdered (Standard Process Inc.). Purchased whole fruits (mango, orange, tangerine and papaya) were peeled, chopped, freeze-dried and ground. The banana was in flour form (Makerere University, Uganda)

to establish pretreatment serum and liver VA concentrations. The remaining gerbils were sorted into seven weight-matched treatment groups ($n = 10$/group) and placed on the customized diets (Figure 1). After a 28-d (Study 1) and 20-d (Study 2) treatment period, the remaining gerbils were killed. Blood was centrifuged at 2200 *g* for 15 min in BD Vacutainer™ Gel and Clot Activator tubes (Becton Dickenson, Franklin Lakes, NJ, USA) for serum isolation. Livers were excised and stored at -80°C.

Study 1 treatments included VA-free basal feed with added WFS or Betanat® or a VA-free customized diet with added powdered brussels sprouts, kale or spinach (Table 1). The WFS and Betanat® contained very concentrated amounts of β-carotene (Table 2) and were mixed into the VA-free basal feed at rates of 0.14 and 0.0097 g/kg feed, respectively. These small masses were weighed with

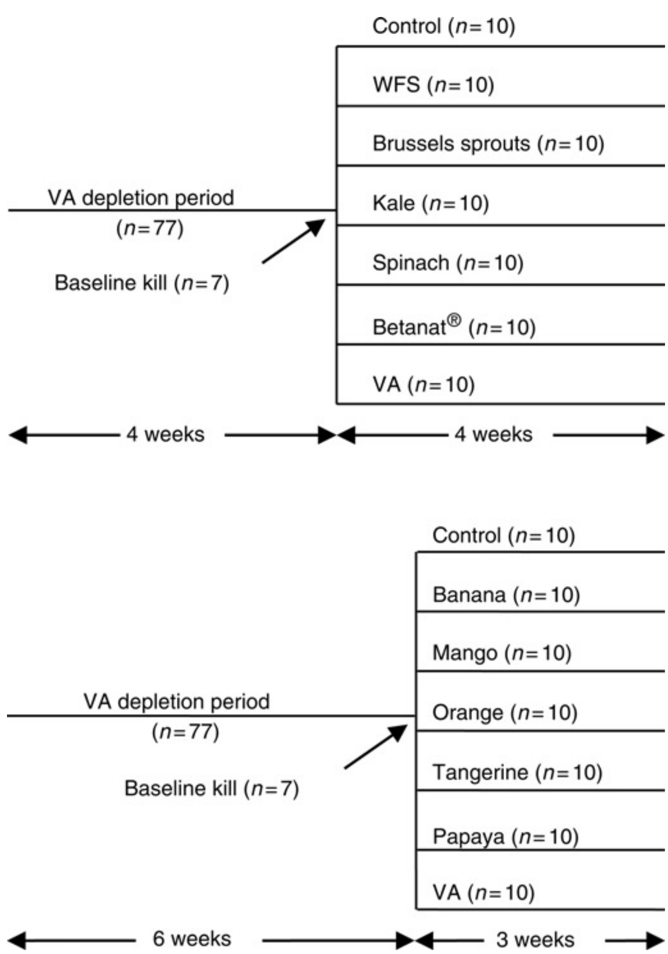

Figure 1 Experimental timeline for two studies to determine the vitamin A (VA) bioefficacy. After a VA depletion period, treatments included VA- and carotenoid-free basal diet with positive and negative controls (VA and control), the WFS or Betanat® (β-carotene from *B. trispora*) (Study 1); and VA- and carotenoid-free custom diets with added powdered brussels sprouts, kale or spinach (Study 1); or banana, mango, orange, tangerine or papaya (Study 2). Diets were equalized to theoretical VA based on 100% bioefficacy of β-carotene equivalents (i.e. 1 mol β-carotene provides 2 mol retinol and 1 mol α-carotene and β-cryptoxanthin provide 1 mol). VA doses for the VA group were matched to the mean theoretical intake of the vegetable groups (Study 1) or half the intake of the fruit groups (Study 2). All treatments included daily doses of cottonseed oil with the volume matched to VA oil intake

a Sartorius CP2P microbalance (Sartorius Corp, Edgewood, NY, USA) that measures to the nearest 0.000001 g. It was assumed that the low component incorporation rates did not affect the macronutrient or fiber content of the feeds. Study 2 treatments included VA-free customized diets with added powdered banana, mango, orange, tangerine or papaya (Table 1). The treatment feeds were mixed to achieve an equalized theoretical VA concentration (Study 1, ~5.2 nmol VA/g feed; Study 2, ~5.8 nmol VA/g feed), assuming 100% bioefficacy (i.e. 1 mol β-carotene provides 2 mol VA and 1 mol α-carotene or β-cryptoxanthin provides 1 mol VA). In Study 1, the source of theoretical VA was solely β-carotene and Study 2 included β-carotene, α-carotene and β-cryptoxanthin.

Both studies employed negative (Control) and positive (VA) control groups, which were fed the VA-free basal feed. The VA group was given twice daily (~5 h apart, Study 1) or once daily (Study 2) doses of VA (as retinyl acetate) dissolved in cottonseed oil. The control and other treatment groups received matched doses of plain cottonseed oil. Feed consumption was measured daily. The amount of VA administered was based on the previous day's intake (Study 1) or half the intake (Study 2) of theoretical VA of the treatment groups.

Carotenoid analysis

Dietary components and feeds were analyzed for carotenoid concentrations using a published procedure.[27] The powdered components were analyzed in triplicate prior to mixing into the feeds. The feeds were analyzed in triplicate at weekly intervals to assess carotenoid degradation. Components or feeds were ground with a mortar and pestle and weighed for analysis (Study 1: 0.02, 0.005, 0.1 or 0.6 g for WFS, Betanat®, vegetable powders or feeds, respectively; Study 2: 0.1, 0.2 or 0.6 g for papaya, orange and tangerine, mango, or banana and feeds, respectively). Study 1 followed the published saponification procedure[27] for all components and feeds. In Study 2, the saponification procedure was modified to maximize provitamin A carotenoid recovery. The banana and mango were saponified as in Study 1. The orange, tangerine and papaya powders were saponified with 750 μL KOH in water (80%) for 30 min (orange and tangerine) or 1.5 h (papaya) at room temperature. The samples were mixed by vortex every five minutes during saponification. Hexane (3 mL) extractions were repeated until the pellet was colorless (5–8 extractions for WFS and Betanat® or 4 extractions for vegetables, fruits and feeds). The combined extracts from the WFS and the Betanat® were brought to 25 or 100 mL, respectively, with hexanes and 1 mL was dried under argon. The entire vegetable, fruit and feed extracts were dried under argon. All samples were reconstituted in methanol:dichloroethane (500 μL, 50:50, v:v) and 50 μL was injected onto a high-performance liquid chromatography (HPLC) system (Waters Corp, Milford, MA, USA). β-*Apo*-8′-carotenyl decanoate was added as an internal standard postsaponification to account for mechanical losses. Lutein, β-carotene, α-carotene and β-cryptoxanthin HPLC-purified external standards were used for identification and quantification. Chromatograms were generated at 450 nm.

Table 2 Carotenoid concentrations in components used to prepare Mongolian gerbil feeds in vitamin A bioefficacy studies

Component	Lutein*,† (nmol/g)	β-Cryptoxanthin (nmol/g)	α-Carotene (nmol/g)	β-Carotene‡ (nmol/g)
Study 1				
WFS§	403.2 ± 54.0	ND**	ND	21,266 ± 250
Brussels sprouts	53.5 ± 1.7	ND	ND	17.0 ± 0.9
Kale	155.2 ± 6.3	ND	ND	17.9 ± 0.3
Spinach	338.7 ± 8.8	ND	ND	70.0 ± 1.8
Betanat®	ND	ND	ND	300,778 ± 277
Study 2				
Banana	3.2 ± 0.2	ND	6.5 ± 0.2	4.7 ± 0.2
Mango	7.7 ± 1.0	ND	ND	77.9 ± 3.2
Orange	8.8 ± 0.6	29.3 ± 1.2	ND	2.7 ± 0.1
Tangerine	5.1 ± 1.0	91.3 ± 6.2	ND	9.8 ± 0.6
Papaya	ND	181.1 ± 6.4	ND	67.7 ± 4.5

ND, not detected; Betanat®, β-carotene from *Blakeslea trispora*

*Values are means ± standard deviation

†The saponification procedure maximized the yield of free β-cryptoxanthin. If saponification time increased beyond 15 min for mango or 30 min for orange and tangerine, free lutein concentration increased as free β-cryptoxanthin decreased. Thus, lutein levels in the fruits could be higher than reported

‡Study 1 reported values include only all-*trans* isomer; Study 2 reported values for banana and mango values include 9-*cis* and 13-*cis* β-carotene isomers. No *cis*-isomers were detected in orange, tangerine and papaya

§WFS: the whole foods supplement contained brussels sprouts, kale and spinach, which were individually tested, and also included carrot powder, acerola powder and calcium stearate

Serum and tissue analyses

All samples were analyzed under gold fluorescent lights to prevent carotenoid photo-oxidation and isomerization. Modified published procedures were used for VA and carotenoid analysis of 500 μL serum[28,29] and ~0.6 g liver.[28] Retinyl butyrate was used as an internal standard to determine extraction efficiency in serum (98 ± 4%) and liver (88 ± 5%) and externally for quantification of retinol and retinyl esters. Dried extracts were reconstituted in methanol:dichloroethane (100 μL, 50:50, v:v) and injected (50 μL) into the HPLC. Livers from the banana and control groups were analyzed for α-retinol using a published procedure.[28]

Statistical analysis and calculations

Values are means ± standard deviation. Data were analyzed using Statistical Analysis System software (SAS Institute Inc, Version 8.2, Cary, NC, USA; 2001). Outcomes of interest (i.e. gerbil weights, feed intakes, serum and liver VA concentrations) were evaluated using analysis of variance at $\alpha < 0.05$ with PROC MIXED, which allows the treatment variances to be different. *Post hoc* differences between groups were determined using Fisher's least significant difference test at $\alpha < 0.05$. β-Carotene to retinol bioconversion factors were calculated using differences in total liver VA in treatment groups compared directly with the VA group. All groups were corrected for the control group total liver VA[28] and utilized a correction factor for the difference in theoretical VA intake between each treatment group.

Results

Carotenoid concentrations

In Study 1, β-carotene and lutein were the two major carotenoids detected in the WFS, brussels sprouts, kale and spinach. β-Carotene was the only carotenoid detected in Betanat®. Lutein concentrations were highest in the WFS, followed by spinach, kale and brussels sprouts. β-Carotene concentrations were highest in Betanat®, followed by WFS, spinach, kale and brussels sprouts. In Study 2, lutein was found in low concentrations in the fruits. β-Cryptoxanthin was not found in banana or mango and was highest in papaya, followed by tangerine and orange. β-Carotene concentration was highest in mango, followed by papaya, tangerine, banana and orange. Banana was the only fruit that contained α-carotene.

The daily theoretical VA intake was calculated as theoretical retinol in the feed times the daily feed intake (Table 3). The theoretical retinol in the WFS and Betanat® feeds was based on premixed values due to the low incorporation rate. These values were also corrected for the β-carotene degradation rate of the vegetable feeds (4.4%), which were re-analyzed during the study. The theoretical retinol in the vegetable and fruit feeds did not differ among the groups (Table 3).

Gerbil weights and intake

Final gerbil weights (Study 1, 73.9 ± 6.4 g; Study 2, 76.1 ± 5.5 g) and liver weights (Study 1, 2.8 ± 0.4 g; Study 2, 3.1 ± 0.9 g) did not differ among treatment groups (Table 4). Feed intake did not differ between groups in Study 1, and ranged from 5.4 ± 0.3 g/d in the WFS and Betanat® groups to 6.0 ± 0.7 g/d in the brussels sprouts group (Table 3). In Study 2, feed intake of the banana group (6.6 ± 1.2 g/d) was significantly higher than the other groups ($P < 0.05$) (Table 3). Theoretical VA intake in Study 1 ranged from 29.8 nmol/d in the Betanat® group to 32.1 nmol/d in the spinach group and differed by ≤5% from the VA group, which was 31.4 nmol retinol/d (Table 3). In Study 2, daily theoretical VA intake of all of the groups varied by ≤5% from twice the daily VA intake of the VA group except the banana group, which took in 12% more VA due to its higher feed intake.

Table 3 Provitamin A carotenoid concentrations of feeds, feed intake and theoretical vitamin A for Mongolian gerbils

Treatment	β-Cryptoxanthin (nmol/g feed*)	α-Carotene (nmol/g feed*)	β-Carotene (nmol/g feed*)	Theoretical vitamin A (nmol/g feed*)	Feed intake (g/d*)	Daily theoretical vitamin A intake (nmol/d)†
Study 1						
Control	ND	ND	ND	0	NM	0
WFS	ND	ND	2.77	5.52	5.4 ± 0.3	29.9
Brussels sprouts	ND	ND	2.54 ± 0.21	5.07 ± 0.42	6.0 ± 0.7	30.4
Kale	ND	ND	2.56 ± 0.20	5.12 ± 0.41	5.9 ± 0.6	30.1
Spinach	ND	ND	2.69 ± 0.13	5.39 ± 0.26	6.0 ± 0.8	32.1
Betanat®	ND	ND	2.77	5.52	5.4 ± 0.3	29.8
VA	ND	ND	ND	0	NM	31.4
Study 2‡						
Control	ND	ND	ND	0	NM	0
Banana	ND	2.17 ± 0.21	1.72 ± 0.23	5.62 ± 0.58	6.6 ± 1.2**	37.5
Mango	ND	ND	2.82 ± 0.25	5.63 ± 0.36	5.6 ± 0.9	31.7
Orange	4.88 ± 0.33	ND	0.50 ± 0.11	5.88 ± 0.41	5.9 ± 0.8	34.7
Tangerine	4.60 ± 0.42	ND	0.53 ± 0.04	5.66 ± 0.43	5.7 ± 0.8	32.1
Papaya	3.34 ± 0.35	ND	1.27 ± 0.12	5.89 ± 0.56	5.6 ± 1.0	32.3
VA§	ND	ND	ND	0	NM	16.7

ND, not detected; NM, not measured; VA, vitamin A; WFS, whole foods supplement; Betanat®, β-carotene from *Blakeslea trispora*
*Values are means ± standard deviation
†Daily theoretical retinol intake was calculated based on mean feed consumption as measured during the study and with the assumption of 100% bioefficacy (i.e. 1 mol β-carotene provides 2 mol retinol and 1 mol β-cryptoxanthin or α-carotene provides 1 mol retinol)
‡The mean feed intake for the banana-fed group is higher than the other groups, ** $P < 0.05$
§Study 2 VA treatment group received half of the theoretical VA of the fruit treatment group's measured mean feed intake × theoretical VA in feed of the previous day

Tissue vitamin A and carotenoid concentrations

Serum retinol concentrations did not differ among treatment groups. Serum retinol ranged from 1.31 ± 0.20 in the kale group to 1.48 ± 0.12 μmol/L in the Betanat® group; and in Study 2, 1.29 ± 0.24 in the VA group to 1.51 ± 0.37 μmol/L in the banana group. Carotenoids were not detected in the serum in either study. As expected in Study 1, liver VA concentration (Figure 2a) and content (Figure 2b) were higher in the VA group than in the other groups ($P < 0.05$). Hepatic VA concentration and content in the control group were lower than the other groups. Hepatic VA concentration of the WFS, brussels sprouts, kale, spinach and Betanat® groups did not differ from the baseline group (Figure 2a). However, on a total liver VA basis, the WFS and kale groups were significantly lower than the baseline group ($P < 0.05$), and the brussels sprouts, spinach and Betanat® groups did not differ from baseline (Figure 2b). *Cis-* and *trans-*β-carotene were detected in the livers (Figure 2c). The Betanat® group had the highest β-carotene content and did not differ from the WFS and spinach groups. The kale group had the lowest β-carotene content and did not differ from the brussels sprouts group.

Table 4 Body and liver weights of Mongolian gerbils in two studies designed to evaluate the vitamin A value of green vegetables and fruits

	Beginning body weight (g)*	Final body weight (g)	Liver weight at kill (g)
Study 1			
Baseline	67.9 ± 12.4†	NA	3.06 ± 0.58
Control	63.5 ± 4.2	75.8 ± 8.5	2.93 ± 0.54
WFS	64.1 ± 4.1	72.0 ± 5.6	2.64 ± 0.28
Brussels sprouts	64.3 ± 4.1	74.6 ± 6.0	2.94 ± 0.45
Kale	64.5 ± 4.2	74.1 ± 5.7	2.82 ± 0.34
Spinach	64.8 ± 4.2	74.7 ± 7.4	2.87 ± 0.43
Betanat®	63.7 ± 4.3	74.4 ± 7.1	2.83 ± 0.54
VA	63.3 ± 4.3	72.0 ± 5.6	2.69 ± 0.27
Study 2			
Baseline	70.3 ± 10.9	NA	3.24 ± 0.75
Control	72.6 ± 4.7	76.6 ± 5.4	2.90 ± 0.41
Banana	71.1 ± 4.6	76.6 ± 4.5	3.29 ± 1.88
Mango	71.4 ± 4.5	75.4 ± 7.6	2.97 ± 0.60
Orange	71.8 ± 4.6	75.9 ± 5.6	3.39 ± 1.36
Tangerine	72.4 ± 4.7	76.3 ± 5.7	2.96 ± 0.38
Papaya	72.3 ± 4.8	75.8 ± 5.0	2.96 ± 0.37
VA	72.9 ± 4.9	76.1 ± 5.6	2.88 ± 0.33

NA, not applicable; VA, vitamin A; WFS, whole foods supplement; Betanat®, β-carotene from *Blakeslea trispora*
*Beginning body weight when allocated to treatment group
†Values are means ± standard deviation. Values do not differ within a study

The hepatic VA concentration and content of the VA group in Study 2 was not higher than most of the fruit groups (Figures 3a and b). On a concentration basis, the hepatic VA of the control group did not differ from baseline (Figure 3a), but the control group did have significantly lower total hepatic VA than baseline (Figure 3b; $P < 0.05$). Total hepatic VA of all of the orange fruits (mango, orange, tangerine and papaya) did not differ from baseline; however, the tangerine group did not differ from the control. Interestingly, the banana group had lower total hepatic VA than the baseline group ($P < 0.05$) and did not differ from the control. Hepatic carotenoids (nmol range) reflected those fed in the feeds (Figure 3c). β-Carotene was detected in all the fruit groups and was highest in mango, followed by papaya and banana, which were followed by tangerine and orange ($P < 0.05$). β-Cryptoxanthin was only detected in orange, tangerine and papaya, and did not differ among these groups. Banana was the only group fed α-carotene and total α-carotene was 1.89 ± 0.35 nmol/liver. Bioconversion of α-carotene was confirmed by quantifying α-retinol in the gerbil livers (0.030 ± 0.003 μmol/liver).

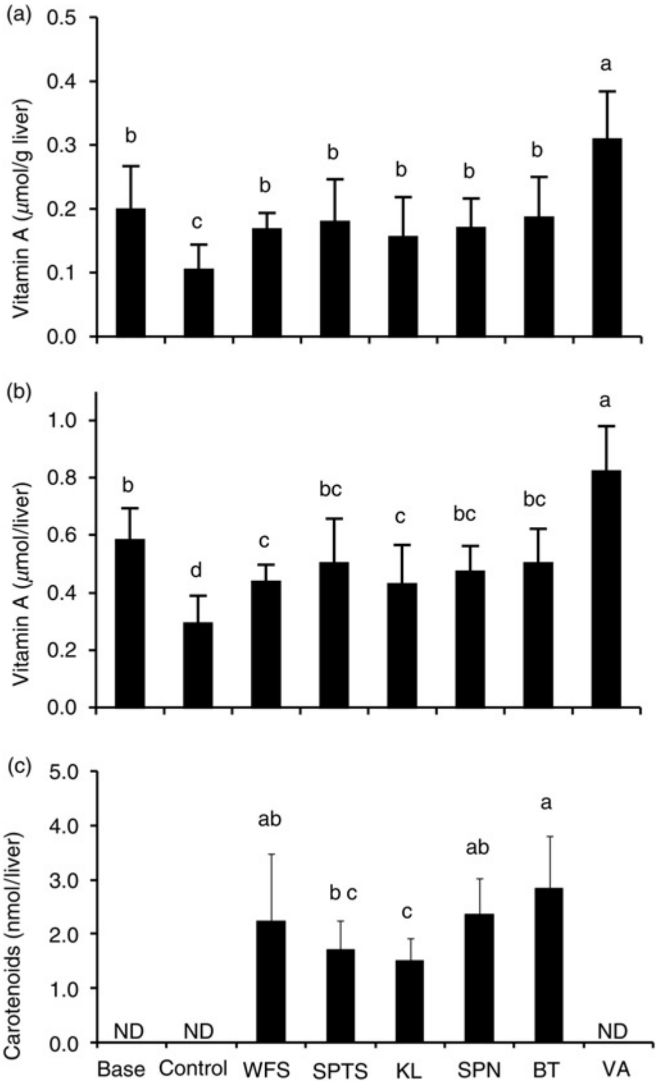

Figure 2 Study 1: Liver vitamin A (μmol) per g liver (a), per liver (b) and total liver β-carotene (c) of Mongolian gerbils at baseline after 4 weeks on a vitamin A-depleted diet (base, $n = 7$) or fed vitamin A and carotenoid-free basal control diet (control), vitamin A and carotenoid-free diet containing a whole foods supplement (WFS), brussels sprouts (SPTS), kale (KL), spinach (SPN) or Betanat® (β-carotene from *B. trispora*) (BT), or vitamin A and carotenoid-free diet supplemented with vitamin A as retinyl acetate in oil (VA) for four additional weeks. Diets were equalized to theoretical vitamin A based on 100% bioefficacy (i.e. 1 mol of β-carotene provides 2 mol vitamin A) of the all-*trans* isomer of β-carotene. Vitamin A intake in the VA group was matched to the mean theoretical vitamin A intake of the WFS, SPTS, KL, SPN and BT groups. Values are means ± standard deviation; $n = 10$. Means with different letters are different, $P < 0.05$. ND, not detected

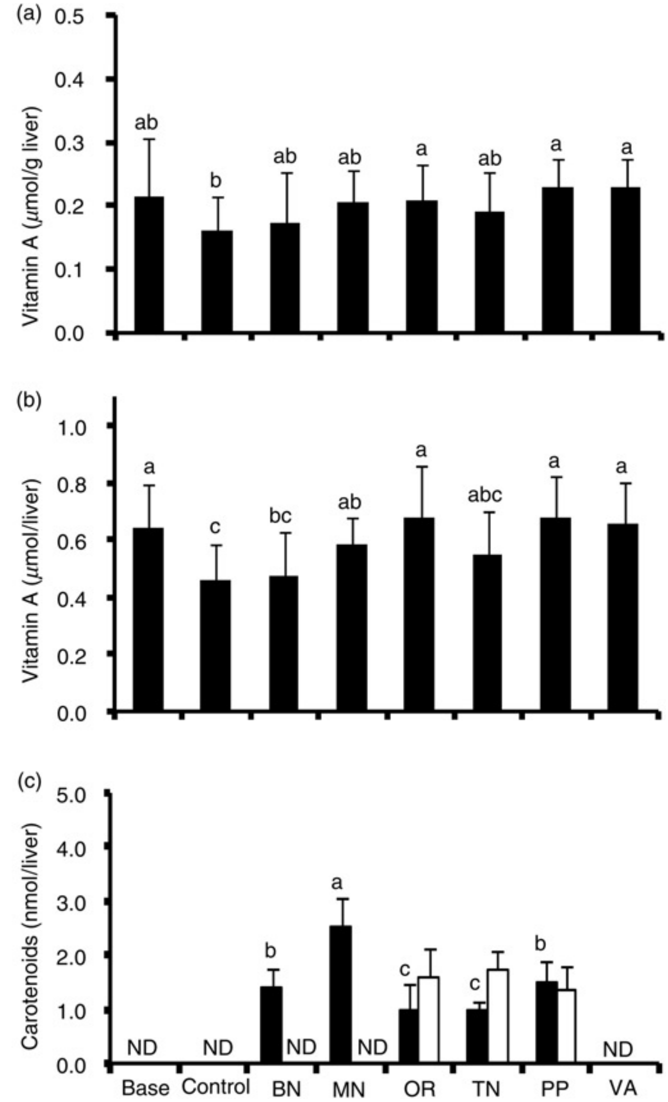

Figure 3 Study 2: Liver vitamin A (μmol) per g liver (a), per liver (b) and total liver β-carotene (■) and β-cryptoxanthin (□) (nmol per liver) (c) of Mongolian gerbils at baseline after six weeks on a vitamin A-depleted diet (base, $n = 7$) or fed vitamin A and carotenoid-free basal control diet (control), vitamin A and carotenoid-free diet containing banana (BN), mango (MN), orange (OR), tangerine (TN) or papaya (PP), or vitamin A and carotenoid-free diet supplemented with vitamin A as retinyl acetate in oil (VA) for three additional weeks. Diets were equalized to theoretical vitamin A based on 100% bioefficacy (i.e. 1 mol of β-carotene provides 2 mol vitamin A, 1 mol of α-carotene or β-cryptoxanthin provides 1 mol vitamin A) of the provitamin A carotenoids. Vitamin A doses in the VA group were matched to half the mean theoretical vitamin A intake of the BN, MN, OR, TN and PP groups. Values are means ± standard deviation; $n = 10$. Means with different letters are different, $P < 0.05$. ND, not detected

Bioconversion factors

Conversion factors were similar in Study 1 and ranged from 2.2 to 3.3 μg β-carotene to 1 μg retinol (Table 5). In Study 2, conversion factors were similar (1.7:1–3.9:1) and overlapped with the vegetables, with the exception of banana (28:1) (Table 5).

Discussion

Fruits and vegetables are rich in carotenoids and provide VA for much of the world's population. Plant foods and supplements made from them may offer a safer alternative to preformed VA and crystalline β-carotene supplements. In these studies, the VA bioefficacy of a WFS and its green vegetable and Betanat® components and five common fruits were directly compared with VA supplements. Three of the WFS components (i.e. brussels sprouts, spinach and Betanat®) and the orange fruits (i.e. mango, orange and papaya) maintained liver VA stores, indicating that these provitamin A sources can prevent VA deficiency in this animal model. Furthermore, the retinol equivalency ratios for the WFS, its components and all of the fruits

Table 5 Bioconversion factors for provitamin A carotenoids (βCE) from a WFS and vegetables (Study 1) or fruits (Study 2) fed to Mongolian Gerbils

	Conversion factor (μg βCE to μg retinol)	Conversion factor (μmol βCE to μmol retinol)
Study 1		
WFS	3.3	1.7
Brussels sprouts	2.3	1.2
Kale	3.2	1.7
Spinach	2.8	1.5
Betanat®	2.2	1.2
Study 2		
Banana	28	15
Mango	2.8	1.5
Orange	1.8	1.0
Tangerine	3.9	2.1
Papaya	1.7	0.9

βCE, β-Carotene equivalents; WFS, whole foods supplement; Betanat®, β-carotene from *Blakeslea trispora*

except banana were very efficient (i.e. 1.7:1–3.9:1 μg β-carotene to 1 μg retinol).

Liver VA is considered the best measure of VA status because the liver is the primary VA storage site.[25] VA deficiency is defined as <0.07 μmol/g liver, and the control group was on the verge of deficiency (0.1 μmol/g liver) in Study 1. The WFS and its green vegetable components, as well as the VA, and Betanat® treatments, were able to prevent this level of VA depletion. Studies by Bulux *et al.*[30] and de Pee *et al.*[31] found that intervention with carrots or dark leafy greens, respectively, were unable to improve VA status as measured by serum retinol concentrations. The lack of an effect in these studies is likely related to the homeostatic control of plasma retinol and the lack of sensitivity of this marker, which has been discussed elsewhere.[32] Recent work utilizing the paired deuterated retinol dilution (DRD) test demonstrated that total body VA stores were maintained by mixed green and yellow vegetables in Chinese kindergarteners[33] and improved by Indian spinach in Bangladeshi men[34] and yellow and green leafy vegetables in Filipino schoolchildren.[35] These latter studies support the current study which found that brussels sprouts and spinach, in addition to Betanat®, were able to maintain VA status as assessed by total liver VA.

The kale and WFS feeds maintained baseline VA concentrations but not total stores. However, they did prevent deficiency when compared with the control and also did not differ from brussels sprouts, spinach or Betanat®. The kale treatment resulted in the lowest hepatic β-carotene which may indicate reduced bioavailability from this vegetable. This may be related to the lower ratio of β-carotene to lutein in kale. In humans, lutein reduced the area under the concentration curve for chylomicron-derived β-carotene and retinyl palmitate.[36] Additionally, the effect of lutein may be more significant when lutein predominates.[37] However, this does not explain the lower total hepatic VA in the WFS compared with baseline and the higher hepatic β-carotene than kale. Novotny *et al.*[38] found that lutein from isotopically labeled kale was more bioavailable than β-carotene in humans. Additional research directly comparing vegetables is needed to discern the effects of carotenoid interactions and food matrices.

In Study 2, the orange and papaya groups had higher hepatic VA concentrations than the control. The orange, tangerine and papaya contained β-cryptoxanthin, which ranged from 72% to 91% of the total provitamin A carotenoids. Polar oxygenated carotenoids such as β-cryptoxanthin demonstrate increased micellarization over neutral hydrocarbons such as β-carotene,[39] thus enhancing bioavailability. Additionally, previous work in Mongolian gerbils suggests that β-cryptoxanthin is as or more efficacious than β-carotene.[40] This does not, however, explain the lack of difference between the tangerine and control groups. While the ratio of β-cryptoxanthin and β-carotene was similar between the orange and tangerine, the carotenoid profile of 32 varieties of these citrus fruits varied enough to distinguish the two fruits.[41] Varietal differences in the *in vitro* bioaccessibility of β-carotene also exist within a fruit species.[42] Considering that climate and geography can influence carotenoid levels in plants,[43] it becomes apparent that VA bioefficacy of plant foods can vary even within a species.

On the basis of total hepatic VA stores in Study 2, all of the orange fruits maintained baseline VA stores. Banana was the only fruit that clearly did not prevent VA depletion. The source of banana for this study was a flour of relatively low provitamin A concentration and required a greater incorporation rate into the gerbil feed, which may have altered the fiber type or content of the feed, although the diet was customized for fiber content. Additionally, banana was the only fruit to contain α-carotene, at a ratio of 1.4:1 with β-carotene. *In vitro* and human studies suggest that α-carotene absorption may be attenuated by the presence of β-carotene.[44,45] However, previous work demonstrated that twice the molar amount of α-carotene maintained VA status as well as β-carotene in VA-depleted gerbils.[28] The presence of measurable amounts of α-retinol and α-carotene in the livers of the gerbils that ate banana confirmed that the α-carotene was bioavailable and that the retinol was utilized for physiological functions. In identically designed studies, the conversion factor for α-carotene was 5.5:1[28] and that of β-cryptoxanthin was 2.74:1.[40] Even though theoretically each provides 1:1, β-cryptoxanthin seems superior.

Bananas are an important food for many people in the world and some reports have suggested that certain cultivars rich in provitamin A carotenoids have the potential to be a major source of VA.[46] The retinol value of β-carotene equivalents in bananas has not previously been evaluated, and was 28:1 in this study. This is seven to 17 times higher than the equivalency ratios for any of the other fruits, a difference that is difficult to explain. From the α-retinol recovery, an equivalency for the α-carotene in the banana can be estimated. In this regard, 0.286 μmol α-carotene was fed during the 20-d treatment period and 0.030 μmol α-retinol was recovered in the liver. Because an equal amount of retinol will be produced from α-carotene cleavage, the equivalency would be 9.5 μmol α-carotene to 1 μmol retinol (18 μg α-carotene to 1 μg retinol). Although this equivalency is still much higher than the other fruits, these data suggest that retinol utilization was higher in the gerbils fed bananas, which is supported by the higher serum retinol concentration in that group,[47] or that absorption was impaired. More research directly comparing different banana cultivars is warranted and complete macronutrient, micronutrient and fiber analysis will be needed[48] to try to discern an 'anti-vitamin A activity factor' to inform current traditional and transgenic breeding efforts with this important staple fruit.[49]

The VA group in Study 2 was fed half the theoretical VA that the fruit groups received. As VA intake and status increase, VA utilization also increases.[50] By halving the VA intake of Study 2's VA group, the utilization rate would better mimic that of the fruit groups and improve the accuracy of the conversion factor. The orange fruits supplied VA as well as the VA group. Specifically, the orange and papaya groups had total hepatic stores slightly higher than the VA group implying that the β-carotene equivalents in these fruits can supply the molar equivalent of VA. Research in humans has demonstrated that fruits are more effective at supplying VA than vegetables.[23,51,52] This may be due to better micellar incorporation of the fruit carotenoids, which are dissolved in oil droplets in chromoplasts. Carotenoids in green leafy vegetables are typically complexed to proteins in chloroplasts.

β-Carotene to VA bioconversion factors for all components tested in this VA-depleted gerbil model ranged from 1.7:1 to 3.9:1 (except for banana). These conversion factors compare with 3.8:1 obtained for Golden Rice[22] and 4.5:1 for spirulina, a green alga used as a high-protein food supplement,[53] both determined using stable isotope techniques in humans. A study utilizing DRD found a conversion factor for Indian spinach of 10:1 in Bangladeshi men[34] and another study that fed intrinsically deuterated spinach to US adults obtained 21:1.[54] Conversion factors are dependent on VA status[55] and the amount of carotenoid consumed.[19] More efficient conversion may also be due to differences in the food matrix, preparation technique or treatment for intestinal helminthes.[34] In addition, the effect of grazing in the current study may have influenced conversion by allowing more efficient absorption of the smaller, more frequent intake of β-carotene. Previous work with VA-depleted gerbils found conversion factors of 2.8:1 for dark-orange maize[27] and 3.5:1 for red carrots.[56] Work with VA-adequate gerbils fed freeze-dried carrots estimated conversion rates to be 9–11:1 for typical orange carrots and ~23:1 for dark-orange carrots containing twice as much β-carotene.[29] These gerbil studies support the finding in Filipino schoolchildren that conversion of carotenoids to VA varies inversely with VA status as measured by three-day DRD.[57]

A recent meta-analysis of supplemental VA and hypervitaminosis A concluded that the physical form of the supplement is a major determinant of toxicity.[6] Water-miscible, emulsified and solid forms of retinol supplements are predicted to be ~10 times more toxic than oil-based forms. While public health supplementation programs that utilize high dose oil-based VA preparations in developing countries appear to be safe in these undernourished populations,[5] it is possible that the use of non-oil-based preformed VA supplementation or fortification may contribute to an increased risk of osteoporosis[7,8] or teratogenicity[6] in VA-sufficient populations. High intakes of β-carotene or other provitamin A carotenoids from foods have never been reported to cause VA toxicity.[5,9] Provitamin A-rich whole food supplements made from green vegetables (e.g. WFS), algae or fruits may provide a safer alternative to preformed VA.

In conclusion, the WFS, its green vegetable components and the orange fruits prevented VA depletion in gerbils. This study demonstrated that β-carotene in a WFS is bioavailable and the VA bioconversion factors for green vegetables and orange fruits are favorable. Additionally, these results suggest that food-based interventions based on fruits and vegetables may be a realistic and sustainable alternative or accompaniment to high-cost synthetic VA supplementation programs designed to overcome VA deficiency globally.

Author contributions: SAA, JAH and SAT participated in the design and technical aspects of the studies. SAA and CRD conducted the studies. SAA and SAT analyzed and interpreted the data, and drafted the manuscript. JAH and CRD provided valuable input into the final manuscript.

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