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Hepatic Vitamin A Concentrations in Vervets (*Chlorocebus aethiops*) Supplemented with Carotenoids Derived from Oil Palm

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Commonly used in biomedical research, vervets (*Chlorocebus aethiops*) are omnivorous but primarily meet their vitamin A requirements from provitamin A carotenoids. Hypervitaminosis A has occurred in vervets that consume feed high in preformed vitamin A. We investigated the vitamin A status of vervets supplemented daily with various antioxidants derived from palm oil. Male vervets ($n = 40$) were placed for 23 wk on a high-fat diet (34.9% energy) containing 645 μg retinol activity equivalents (RAE), with 515 μg RAE from preformed vitamin A. Vervets were randomized to 5 treatments (duration, 20 mo): control; 100 mg d-α-tocopheryl acetate; 100 mg oil palm (*Elaeis guineensis*)-derived vitamin E; 50 mg oil palm-derived vitamin E + 50 mg carotenoid complex + unrestricted palm-derived water-soluble antioxidants; and 5) unrestricted water-soluble antioxidants. Livers ($n = 38$) were analyzed for vitamin A, α-retinol (α-vitamin A), and carotenoids. Median hepatic vitamin A and total carotenoid concentrations were 6.49 μmol/g and 4.30 nmol/g, respectively. Compared with controls, vervets fed the carotenoid complex had higher hepatic vitamin A (11.9 ± 5.1 μmol/g), α-vitamin A (1.3 ± 0.7 μmol/g), α-carotene (11.5 ± 5.3 nmol/g), β-carotene (15.6 ± 8.6 nmol/g), and total carotenoids (28.1 ± 13.9 nmol/g) but lower lutein (0.66 ± 0.28 nmol/g) and zeaxanthin (0.24 ± 0.06 nmol). NHP may benefit from replacement of preformed vitamin A with carotenoids in feeds; however, bioconversion efficiency in these models should be investigated to determine optimal levels.

Abbreviations: αC, α-carotene; αRE, α-retinyl ester; βC, β-carotene; BCO1, β-carotene 15, 15′-oxygenase; HFD, high-fat diet; OPC, oil palm carotenoids; RAE, retinol activity equivalents; RE, retinyl esters.

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Vitamin A is a fat-soluble vitamin important for normal growth and development, immune function, and vision.[35] Preformed vitamin A (that is, retinol and retinyl esters [RE]), can be obtained by consuming animal-sourced foods, fortified foods, and supplements.[35] When consumed in amounts exceeding daily needs, vitamin A is stored primarily in the liver. The central cleavage of the provitamin A carotenoids, such as α-carotene (αC), β-cryptoxanthin, and β-carotene (βC), by the cytosolic enzyme β-carotene-15, 15′-oxygenase (BCO1) yields 1, 1, and 2 molecules of retinal, respectively, which is subsequently reduced to retinol. BCO1 and scavenger receptor B1 are regulated by dietary vitamin A by a negative feedback loop involving the transcription factor intestine-specific homeobox.[26,43]

Vervets (*Chlorocebus aethiops*) are commonly used in biomedical research[2] because they are not endangered, are easily handled, are a good model for hypertension,[8] and have been used in studying the effects of dietary lipids on plasma lipoprotein metabolism and atherosclerosis.[1,53,54] Unlike other NHP species, they have done relatively well in weathering the loss of habitat perhaps due to their ability to adapt their diets to suit their environment,[18] such as raiding vegetable, cereal, and fruit crops.[40] Vervets are naturally omnivorous, consuming leaves, seeds, grasses, fruits, bird eggs, birds, lizards, rodents, and invertebrates, with a preference for fruits and flowers.[10,19]

The vitamin A requirements of vervets and other NHP have not been defined precisely and are based partly on the Institute of Medicine's estimated average daily requirement for adult male humans (625 μg retinol activity equivalents [μg RAE]).[20] Diets containing 10,000 IU (3000 μg RAE as preformed vitamin A) per kilogram of dry matter are generally considered to be adequate; however, little evidence is available to justify this concentration.[34] Very high vitamin A hepatic stores have been reported in NHP consuming common feeds formulated with preformed vitamin A.[31,37,38] Considering that the bioconversion of provitamin A carotenoid to vitamin A is regulated in humans (and presumably in NHP species as well) and that free-living vervets likely consume most of their vitamin A as provitamin A carotenoids in nature, some authors suggest that providing vitamin A as carotenoids in feeds may be safer.[5] Moreover, the potential effects of hypervitaminotic but subtoxic vitamin A hepatic stores on biomarkers of status and physiology, such as elevated circulating RE and catabolism, are largely unknown.

Here we investigated the vitamin A status of vervets on a high-fat diet (HFD) supplemented with various antioxidant preparations derived from the palm fruit, one of which included a natural mixed carotenoid complex, for an extended period. Carotenoid intake may protect against many chronic diseases, including atherosclerosis.[6,21] Liver samples from these vervets were analyzed for vitamin A, α-retinol species (α-vitamin A), and carotenoid concentrations to further investigate the vitamin A status of NHP housed for biomedical research. In addition

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to βC, red palm oil contains αC that produces α-retinol upon central cleavage in addition to retinol.

Materials and Methods

Research animals. Adult male vervets (*Chlorocebus aethiops*; n = 40 total) were recruited from the South African Medical Research Council Primate Unit's inhouse breeding colony; 30 of these animals were bred inhouse, and the remaining 10 originally were wild-caught with permission from Nature Conservation as part of a study examining the effect of dietary antioxidants on the progression of atherosclerosis. The treatment protocols were reviewed and approved by the Ethics Committee for Research on Animals of the South African Medical Research Council. The holding facilities and animal health and welfare were inspected twice annually by the local Society for the Prevention of Cruelty to Animals. In addition, the Ethics Committee performed postapproval monitoring ensuring adherence to the procedures and activities outlined in the study protocol.

Vervets were housed in a closed sterile environment with 15 to 20 air changes hourly. The room air temperature was maintained at 23 to 26 °C, with 45% to 50% humidity and a 12:12-h photoperiod; fresh water was available automatically and without restriction. For the duration of the study, housing was in single cages (900 × 700 × 1200 cm), which are fitted with full perches and mesh grooming panels to allow social interaction with adjacent animals. In addition, each vervet had access to a large exercise cage for 24 h weekly. Separate foraging devices were offered 3 times each week to encourage foraging. All procedures carried out with the vervets, including handling and care, were performed by the same qualified people. All vervets were housed according to the Primate Unit's standard operating procedures and in accordance with the revised South African National Standard for the Care and Use of Animals for Scientific Purposes.[45] The Primate Unit at the South African Medical Research Council is accredited by the South African Veterinary Council (registration no. FR15/13973), and its research and veterinary care are regulated by the South African Bureau of Standards to ensure compliance with legislation and international guidelines for animal welfare.

Experimental feeds. Typical experimental feed details have been published elsewhere.[53] Briefly, all 40 vervets were placed on a high-fat diet (HFD; 34.9% energy from fat), beginning in December 2003, for 23 wk until baseline (May 2004) as a run-in period to acclimate them to procedures. The HFD was formulated inhouse from household ingredients and vegetables; including bananas, lard, margarine, butter, beef mince, chicken with skin, full-cream milk powder, egg, rice, dried beans, cake flour, sugar, salt, cabbage, potato, carrot, and rice (Figure 1). HFD ingredients were baked into 'patties' (92.2 ± 6.7 g each) and stored at –20 °C until use. Patties were served immediately after thawing at room temperature; vervets received one patty twice daily. Vervets typically consumed the entire meal, with minimal wastage on high-fat formulated diets (between 4.1% and 6.1%).[54] In addition, the HFD was supplemented daily with minerals and vitamins to optimize the micronutrient intake of the vervets. A piece of raw apple (approximately 70 g) was supplied daily to each vervet as part of the foraging enrichment. Suppliers of the food ingredients were kept constant.

Vervets were randomized to 1 of 5 (n = 8 per group) experimental treatments, with 2 of the wild-caught vervets assigned to each group: 1) HFD control (no supplements), 2) HFD + 100 mg d-α-tocopheryl acetate (commercial product; Hoffman-La Roche, Basel, Switzerland) daily, 3) HFD + 100 mg vitamin E derived from oil palm (Tocomin 50% oil suspension; natural full-spectrum tocotrienol–tocopherol complex; Carotech, Naples, FL) daily, 4) HFD + 50 mg oil palm vitamin E + 50 mg oil palm-derived carotenoid complex (OPC; Caromin 20% concentrate, Carotech; natural mixed carotenoid complex consisting mainly of αC, βC, and lycopene with approximately 240,000 IU vitamin A/g) daily + free-choice oil-palm–derived water-soluble antioxidants (as an *E. guineensis* juice) provided after thawing from the frozen state in addition to the drinking water supply, and 5) HFD + free-choice oil-palm–derived water-soluble antioxidants as undiluted palm juice (as for treatment 4) in addition to the water supply. Vervets consumed these treatments for 20 mo. For experimental patties containing commercial vitamin E, oil palm-derived vitamin E, and OPC, supplements were mixed with the fat portion of the food before incorporation into the full recipe to ensure optimal mixing. Water-soluble supplements were chosen because they could be purified and recovered from the palm-waste manufacturing process. Palm juice is a water-soluble extract rich in phenolic acids recovered from the vegetation liquor generated from the milling of oil-palm fruits[46] and contains substantial amounts of caffeic, *p*-hydroxybenzoic, and caffeoylshikimic acids.[41] For treatments 4 and 5, palm juice was initiated 1 wk after initiation of study treatments. Palm juice, Tocomin 50%, and Carmin 20% (both from Carotech) were couriered to the South African Medical Research Council in batches as needed. Palm juice was stored at –20 °C and thawed as needed, whereas the fat-soluble supplements were kept at 4 °C until used to formulate study treatments.

	Amount (g) per 2 patties	Proportion (%) of feed
Milk powder, full cream	5.6	2.2
Cake flour	31.5	12.7
Sugar	10.0	4.0
Salt	1.3	0.5
Dicalcium phosphate	1.4	0.6
Eggs, large, raw	35.3	14.3
Butter, melted	4.7	1.9
Margarine, melted	4.7	1.9
Lard	5.0	2.0
Chicken, with skin	10.0	4.0
Dry white beans	20.9	8.5
Cabbage	23.9	9.7
Carrots	15.0	6.1
Potatoes, without skin	18.5	7.5
Rice, white	20.0	8.1
Regular minced beef	22.5	9.1
Banana, no peels	16.8	6.8
Total	247.1	100%

Figure 1. Composition of the high-fat diet composition. Batches were mixed and baked into patties and stored at -20°C until use. Adult male vervets (*Chlorocebus aethiops*) consumed 2 patties daily.

Dietary vitamin A and provitamin A carotenoid content. The Medical Research Council's Food Composition Tables[25] were used to compile the diets and calculate the quantities and nutrient energy of the experimental HFD. Estimates of vitamin A and carotenoid contents were calculated by using ingredient equivalents compiled from the US Department of Agriculture's National Nutrient Database for Standard Reference Release 28 (Table 1).[51] The HFD contained 645 µg RAE per vervet daily (amount in 2 patties), with 145 µg as preformed vitamin A from the food and 370 µg as preformed vitamin A from the vitamin mix (thus, 515 µg RAE as total preformed A each day). The HFD provided 1290 µg βC, 530 µg αC, 3 µg β-cryptoxanthin, and 240 µg lutein + zeaxanthin daily.

Table 1. Dietary vitamin A (VA) and carotenoid content as estimated by ingredients in the USDA National Nutrient Database for Standard Reference Release 28[a]

	Database no.	Amount (µg, except VA [RAE]) in 100 g of ingredient						Amount (µg, except VA [RAE]) per vervet daily (2 patties)					
		Retinol[b]	VA	βC	αC	βCX	L+Z	Retinol[b]	VA	βC	αC	βCX	L+Z
Milk, dry, whole, without added vitamin D	01212	253	258	55	0	0	0	14	14	3	0	0	0
Wheat flour, white, cake, enriched	20084	0	0	0	0	0	3	0	0	0	0	0	1
Granulated sugar	45105765	0	0	0	0	0	0	0	0	0	0	0	0
Salt	45079336	0	0	0	0	0	0	.	.	.	.	.	.
Egg, whole, raw, fresh	01123	160	160	0	0	9	503	57	57	0	0	3	178
Butter, salted	01001	671	684	158	0	0	0	32	32	7	0	0	0
Margarine, regular, hard, soybean (hydrogenated)	04073	768	819	610	0	0	0	36	39	29	0	0	0
Lard	04002	0	0	0	0	0	0	0	0	0	0	0	0
Chicken, skin (drumsticks and thighs), cooked, roasted	05675	54	54	0	0	1	91	5	5	0	0	0	9
White kidney beans	45122317	.	0	.	.	.	.	.	0	.	.	.	.
Cabbage, raw	11109	0	5	42	33	0	30	0	1	10	8	0	7
Carrots, raw	11124	0	835	8285	3477	0	256	0	125	1240	520	0	38
Potatoes, boiled, cooked without skin, flesh, without salt	11367	0	0	1	0	0	9	0	0	0	0	0	2
Rice, white, medium-grain, cooked, unenriched	20451	0	0	.	.	.	.	0	0	.	.	.	.
Beef, ground, unspecified fat content, cooked	23220	7	7	0	0	0	0	2	2	0	0	0	0
Bananas, raw	09040	0	3	26	25	0	22	0	1	4	4	0	4
Total		1913	2825	9177	3535	10	914	145	275	1293	532	3	239

αC, α-carotene; βC, β-carotene; βCX, β-cryptoxanthin; L+Z, lutein and zeaxanthin
[a]Available at: https://ndb.nal.usda.gov/ndb/
[b]The vitamin mix added to the patties contained an additional 370 µg preformed vitamin A.

Euthanasia and sample collection. Vervets were euthanized in May 2006 according to standard, routine necropsy procedures at this facility.[12,13,15,54] After premedication (such as that used for routine blood sampling: 10 mg/kg IM ketamine), animals were deeply anesthetized by using 2.5% fluothane and 1% oxygen. During exsanguination, the vasculature was flushed with heparinized saline. Liver samples (site not specified) were collected before perfusion fixation, frozen at –80 °C, and shipped on dry ice to the Department of Nutritional Sciences at the University of Wisconsin-Madison, where they were stored at –80 °C until analysis. For perfusion fixation, fixative containing 1% glutaraldehyde and 4% formaldehyde in 0.2 M phosphate buffer (pH 7.2) was applied through the left ventricle at physiologic pressure for 30 min.

One vervet in the group fed oil palm vitamin E and one in the group fed commercial vitamin E were euthanized at 4 and 7 mo after baseline, respectively, due to a 'twisted colon;'[14] a liver sample for the vervet in the oil palm vitamin E group was not obtained. In addition, one vervet was euthanized 11 mo after baseline due to weight loss resulting from a kidney cyst discovered postmortem; a liver sample was not collected. Therefore, the control and oil palm vitamin E treatment groups each had 7 vervets, bringing the final number analyzed to 38.

Extraction and detection of hepatic vitamin A and α-vitamin A species. Samples were thawed twice: once for analysis of retinol and RE by HPLC and then for concurrent analysis by ultraHPLC and HPLC for optimization of α-retinyl esters (αRE) and carotenoids, respectively. α-Retinol was detected in the first HPLC analysis, but the HPLC method was not optimized for quantification of α-retinol. Therefore, samples were extracted again and analyzed by using ultraHPLC for α-vitamin A because of the high amounts of αC in treatment 4. Liver samples were extracted as described previously,[49] with minor modifications. Briefly, 100 mg liver tissue, collected from different locations of the provided sample, was ground with approximately 5 g anhydrous sodium sulfate. C23 β-*apo*-carotenol was added as an internal standard, and the sample was repeatedly extracted with dichloromethane and filtered into a 50-mL volumetric flask. One mL extract was dried under nitrogen and resuspended in 200 µL 50:50 (v:v) methanol:dichloroethane; 1 µL was then injected into an ultraHPLC H-class system with a photodiode array detector and an HSS C_{18} column (2.1 × 150 mm; 1.8 µm; Acuity, Waters, Milford, MA). Samples were eluted at 0.4 mL/min with 70:25:5 (v:v:v) acetonitrile:water:2-propanol with 10 mM ammonium acetate (solvent A) and 75:25 (v:v) methanol:2-propanol (solvent B) using the following gradient: 7 min hold at 100% A, 4-min linear gradient to 95% B, 12-min linear gradient to 99% B, 2-min reverse gradient to 100% A, and then a 4-min hold at 100% A for reequilibration. Chromatograms were generated at 325 and 311 nm to quantify RE and αRE, respectively. RE and αRE were verified by using HPLC-purified standards isolated from pig and rat livers (animals previously dosed with

α-retinyl acetate). Retinyl oleate and retinyl palmitate, as well as α-retinyl oleate and α-retinyl palmitate, could not be resolved and are reported together.

Extraction and detection of hepatic carotenoids. Liver (300 mg) was extracted and reconstituted as described earlier, except that 200 µL *trans*-β-*apo*-8′-carotenal (Sigma, St Louis, MO) was used as internal standard. Carotenoids were analyzed as described previously[3] with modifications. Briefly, 50 µL was injected into a reversed-phase HPLC system (Waters) consisting of a guard column, 5-µm YMC carotenoid column (250 mm × 4.6 mm), binary pump (model 1525), and a 2996-photodiode array detector. Samples were eluted by using the same gradient method as described previously.[3] Chromatograms were obtained at 450 nm to quantify carotenoids, which were confirmed by using HPLC-purified standards.

Statistical analyses. Unless otherwise noted, evaluation of model assumptions and statistical analyses were performed by using SAS (version 9.4; SAS Institute, Cary, NC). Normality of residuals and the homogeneity of variance were determined by using the Shapiro–Wilk (univariate procedure) and Levene tests, respectively. Data satisfying these assumptions were analyzed by one-factor ANOVA by using the generalized linear model procedure with subsequent Fisher protected Least Significant Difference (LSD) tests for group comparisons when variables were compared among treatments. When residuals were not normally distributed, the data were log-transformed (or an inverse was taken, as with weight) and analyzed as described. If transformed residuals were not normally distributed, the original data were analyzed by using a nonparametric test on ranked data. When residuals were normally distributed but Levene testing revealed nonconstant variance, P values were obtained from Welch ANOVA. The mixed procedure was used to assess a potential association with hepatic vitamin A by using diet as a categorical variable because provitamin A intake was different for the OPC group. As for other analyses, when residuals were not normally distributed, data were transformed (logarithm: hepatic carotenoid or vitamin A species, inverse: weight). A P value of 0.05 or less was considered significant. Baseline liver samples were not available; therefore, ending weight and change in weight across the study were compared with liver vitamin A concentrations. In the text, summarized data are reported as mean ± 1 SD, whereas data in tables are reported as medians (1st, 3rd quartiles) unless otherwise noted, because several variables had nonnormally distributed residuals. Data in tables are presented in original (nontransformed) form; however, where noted, group comparisons were performed on transformed or ranked data.

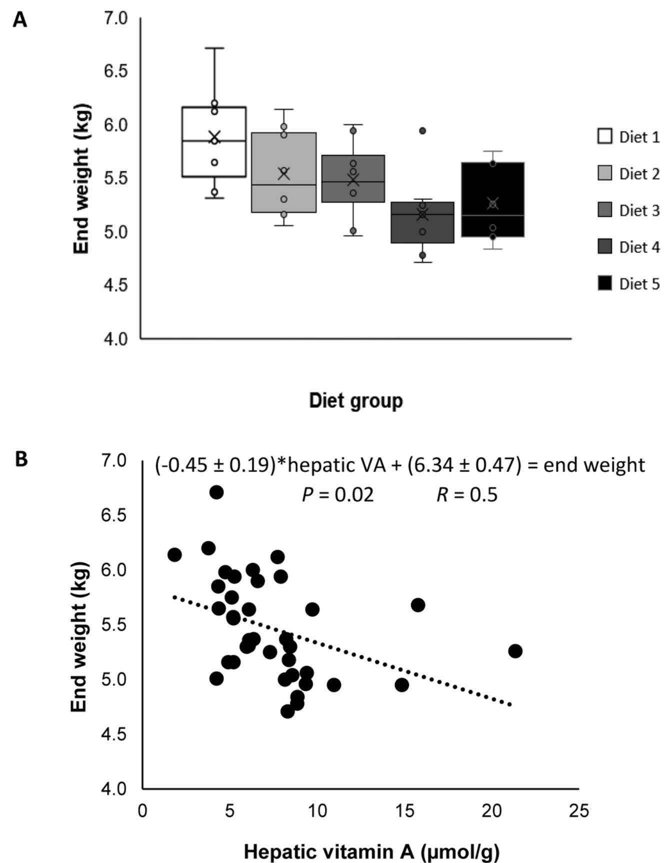

Figure 2. (A) Box plots of end weights (kg) of adult male vervets (*Chlorocebus aethiops*; n = 39) in 5 diet groups, which did not differ (P = 0.1). Left to right, high-fat diet was supplemented with: 1) no supplements (control); diet 2) free-choice palm-derived water-soluble antioxidants; 3) 100 mg d-α-tocopheryl acetate; 4) 100 mg oil palm (*Elaeis guineensis*)-derived vitamin E; and 5) 50 mg oil palm vitamin E + 50 mg oil-palm–derived carotenoid complex + free-choice palm-derived water-soluble antioxidants. (B) Weight of adult male vervets (n = 38) was negatively associated (P = 0.02) with hepatic vitamin A (µmol/g) concentration. P values reflect analyses with transformations (A, 1/kg; B, log[vitamin A]) to address nonconstant variance.

Results

Baseline characteristics. Vervets were 6.46 ± 1.33 y old (mean ± 1 SD) and weighed 5.58 ± 0.53 kg at the initiation of experimental treatments (baseline). Age and weight did not differ by treatment group (P = 0.8 and P = 0.1, respectively), although vervets given oil palm vitamin E and OPC appeared to weigh less at the end of the study (Figure 2 A). Relative to baseline weights, vervets lost 0.13 ± 0.36 kg over the study, and weight loss did not differ by diet group (P = 0.9).

Hepatic vitamin A and α-vitamin A. Hepatic vitamin A concentration, defined as retinol + RE, ranged from 1.9 to 21.4 µmol/g for individual vervets. Thus, all vervets had hypervitaminosis A, defined in humans as 1 µmol/g or greater.[50] Overall, the hepatic concentration of vitamin A (retinol + RE; mean ± 1 SD) was 7.6 ± 3.8 µmol/g liver and was significantly (P < 0.05) different among treatment groups (Table 2). Hepatic free-retinol concentrations were not different among groups; however, individual and summed RE concentrations were higher (P < 0.05) in the OPC group compared with other treatment groups (Table 2). Weight at the end of the study was negatively associated with hepatic vitamin A concentrations after accounting for the effect of diet (P = 0.02, log-transformed to address nonconstant variance; Figure 2 B); however, the change in weight (at study end – baseline) was not significant after accounting for the effect of diet (P = 0.9).

Total hepatic α-vitamin A species ranged from 0.10 to 2.7 µmol/g, with a mean of 0.5 ± 0.5 µmol/g (Table 2). All verified αRE as well as the ratio of α-vitamin A to vitamin A were higher (P < 0.05) in the OPC group compared with other treatment groups. In addition, hepatic concentrations of free α-retinol were higher in the OPC group than all other treatment groups (Table 2).

Overall, the concentration of total hepatic vitamin A and α-vitamin A species was 8.7 ± 4.6 µmol/g (mean ± 1 SD), with values ranging from 1.98 to 25.5 µmol/g for individual vervets. Vervets given the OPC supplement exhibited the highest hepatic concentrations of vitamin A among all groups (P < 0.05; Table 2).

Hepatic carotenoids. Total hepatic carotenoid concentration ranged from 0.6 to 53.2 nmol/g, with a mean of 9.02 ± 11.7 nmol/g for all treatment groups (Table 3). Hepatic concentrations of αC

Table 2. Hepatic vitamin A (VA) and αVA concentration (μmol/g liver) of adult male vervets (*Chlorocebus aethiops*; $n = 38$) enrolled in an antioxidant study

	Control (no supplement) ($n = 7$)	Commercial vitamin E ($n = 8$)	Oil-palm–derived vitamin E ($n = 7$)	Oil-palm–derived vitamin E + carotenoids + antioxidants ($n = 8$)	Palm-derived antioxidants ($n = 8$)	Total ($n = 38$)	*P*
Vitamin A							
Retinol	0.33 (0.26, 0.46)	0.40 (0.24, 0.54)	0.44 (0.42, 0.54)	0.52 (0.33, 0.58)	0.32 (0.26, 0.39)	0.40 (0.27, 0.56)	0.5
Retinyl laurate[d]	0.15 (0.14, 0.26)[c]	0.26 (0.19, 0.37)[b,c]	0.35 (0.28, 0.36)[b]	0.51 (0.45, 0.70)[a]	0.27 (0.15, 0.30)[b,c]	0.29 (0.20, 0.39)	0.0009
Retinyl oleate+retinyl palmitate[e]	3.51 (3.40, 4.86)[b]	5.12 (4.63, 6.09)[b]	6.29 (4.76, 6.81)[b]	8.14 (6.96, 12.19)[a]	4.45 (4.03, 5.60)[b]	5.25 (4.08, 6.88)	0.002
Retinyl stearate[e]	0.47 (0.41, 0.68)[b]	0.62 (0.59, 1.00)[b]	0.84 (0.65, 0.90)[b]	1.25 (1.06, 1.61)[a]	0.65 (0.51, 0.76)[b]	0.68 (0.57, 1.03)	0.0008
Total retinyl esters[e]	4.11 (3.97, 5.80)[b]	5.93 (5.49, 7.46)[b]	7.53 (5.75, 7.98)[b]	9.83 (8.47, 14.50)[a]	5.28 (4.82, 6.68)[b]	6.12 (4.93, 8.26)	0.001
Total VA (all)[e]	4.38 (4.29, 6.22)[b]	6.22 (5.88, 8.00)[b]	8.16 (6.29, 8.39)[b]	10.35 (8.80, 15.09)[a]	5.60 (5.09, 7.05)[b]	6.49 (5.20, 8.55)	0.002
α-Vitamin A							
α-Retinol[f]	0.01 (0.00, 0.01)[b]	0.02 (0.01, 0.02)[b]	0.01 (0.01, 0.02)[b]	0.04 (0.03, 0.05)[a]	0.01 (0.01, 0.01)[b]	0.01 (0.01, 0.03)	0.004
α-Retinyl oleate+α-retinyl palmitate[e]	0.22 (0.20, 0.25)[c]	0.32 (0.28, 0.36)[b,c]	0.37 (0.28, 0.44)[b]	0.87 (0.77, 1.42)[a]	0.29 (0.27, 0.32)[b,c]	0.30 (0.23, 0.46)	<0.0001
α-Retinyl stearate[d,f]	0.01 (0.01, 0.02)[c]	0.02 (0.01, 0.04)[b,c]	0.04 (0.03, 0.05)[b]	0.13 (0.10, 0.18)[a]	0.03 (0.02, 0.03)[b]	0.03 (0.01, 0.05)	<0.0001
Total α-retinyl esters[e]	0.22 (0.20, 0.27)[c]	0.35 (0.31, 0.38)[b,c]	0.41 (0.30, 0.51)[b]	1.00 (0.87, 1.61)[a]	0.32 (0.29, 0.35)[b,c]	0.34 (0.26, 0.52)	<0.0001
Total α-VA (all)[e]	0.23 (0.20, 0.29)[c]	0.35 (0.33, 0.39)[b,c]	0.43 (0.31, 0.52)[b]	1.04 (0.90, 1.65)[a]	0.33 (0.29, 0.36)[b,c]	0.35 (0.28, 0.53)	<0.0001
Total VA and α-VA species[e]	4.82 (4.69, 6.81)[b]	6.95 (6.65, 9.01)[b]	9.08 (7.12, 9.43)[b]	12.05 (10.22, 17.70)[a]	6.28 (5.62, 7.86)[b]	7.20 (5.86, 9.81)	0.009

Data are reported as median (1st quartile, 3rd quartile).
Different superscript letters within a row denote significant differences between groups (one-factor ANOVA with Fisher protected least significant difference testing or a nonparametric [ranked] test), with a > b > c. *P* values result from testing the null hypothesis that all groups are not statistically different for a given variable.
[d]Nonnormally distributed residuals; *P* value reflective of a non-parametric (ranked) analysis. The original (untransformed) data are reported here.
[e]Statistical analysis carried out on the log transform of the variable, that is, log(y). The original (untransformed) data are reported here.
[f]Not all species were detected in all samples, therefore the number reported varies from 5 to 8.

Table 3. Hepatic carotenoid concentration (nmol/g liver) of adult male vervets (*Chlorocebus aethiops*; $n = 38$)

	Control (no supplement) ($n = 7$)	Commercial vitamin E ($n = 8$)	Oil-palm–derived vitamin E ($n = 7$)	Oil-palm–derived vitamin E + carotenoids + antioxidants ($n = 8$)	Palm-derived antioxidants ($n = 8$)	Total ($n = 38$)	*P*
Lutein	1.47 (1.36, 1.96)[a]	1.83 (1.41, 2.40)[a]	1.59 (1.34, 2.11)[a]	0.67 (0.49, 0.87)[b]	1.74 (1.67, 1.96)[a]	1.54 (0.99, 2.06)	0.003
Zeaxanthin[a]	0.45 (0.40, 0.74)[a]	0.54 (0.45, 0.63)[a]	0.48 (0.42, 0.59)[a]	0.25 (0.19, 0.29)[b]	0.46 (0.41, 0.56)[a]	0.44 (0.33, 0.59)	0.004
α-Carotene[b]	0.62 (0.50, 0.79)[c]	0.77 (0.55, 0.97)[c]	1.27 (1.03, 1.66)[b]	9.69 (8.06, 15.42)[a]	0.84 (0.67, 1.10)[c]	1.00 (0.67, 1.83)	<0.0001
All *trans*-β-carotene[a]	0.26 (0.22, 0.37)[c]	0.34 (0.24, 0.43)[c]	0.56 (0.46, 0.91)[b]	7.78 (6.42, 13.99)[a]	0.37 (0.26, 0.40)[c]	0.43 (0.27, 0.91)	<0.0001
β-carotene + isomers[a]	0.43 (0.37, 0.58)[c]	0.53 (0.43, 0.70)[c]	0.91 (0.75, 1.34)[b]	11.7 (10.4, 21.2)[a]	0.60 (0.42, 0.69)[c]	0.70 (0.45, 1.38)	<0.0001
Total hepatic carotenes[b]	1.02 (0.88, 1.37)[c]	1.30 (0.98, 1.67)[c]	2.17 (1.79, 3.00)[b]	21.0 (19.1, 37.7)[a]	1.44 (1.08, 1.79)[c]	1.69 (1.12, 3.18)	<0.0001
Total hepatic carotenoids[b]	3.45 (2.70, 4.43)[b]	3.84 (3.13, 4.29)[b]	4.15 (3.74, 5.32)[b]	22.2 (19.9, 38.3)[a]	3.67 (3.32, 4.47)[b]	4.30 (3.35, 6.86)	<0.0001

Data are reported as median (1st quartile, 3rd quartile).
Different superscript letters within a row denote significant ($P < 0.05$, one-factor ANOVA or chi-squared testing) differences between groups; a > b > c. *P* values result from testing the null hypothesis that all groups are not statistically different for a given variable.
[a]Statistical analysis done on the log transform of the variable, that is, log(y). The original (untransformed) data are reported here.
[b]Nonnormally distributed residuals; *P* value reflective of a nonparametric (ranked) analysis. The original (untransformed) data are reported here.

and βC, 9-*cis*- and 13-*cis*-βC, total carotenoids, and carotenes were higher in the OPC group compared with all other treatment groups (Table 3). After the effect of diet was included, hepatic carotenoids (log-transformed) were positively associated with hepatic vitamin A + α-vitamin A ($P = 0.04$; $R^2 = 0.80$); the same was true for hepatic carotenes (log-transformed; $P = 0.04$; $R^2 = 0.87$). Mean total hepatic βC (all-*trans* + 9-*cis* + 13-*cis*) concentration for the OPC group was 15.6 ± 8.6 nmol/g liver, which was significantly ($P < 0.0001$) higher than in all other groups. Interestingly, hepatic concentrations of lutein and zeaxanthin were lowest in the OPC group (Table 3). Hepatic carotenoid concentration varied greatly among individual vervets in the OPC group; however, coefficients of variation were similar to those in other groups, with a range of 27% to 55% among carotenoids in the OPC group. Hepatic carotene and carotenoid concentration appeared to increase with total hepatic vitamin A (vitamin A+α-vitamin A) concentration (Figure 3 A). After the effect of diet was included, hepatic lutein was associated

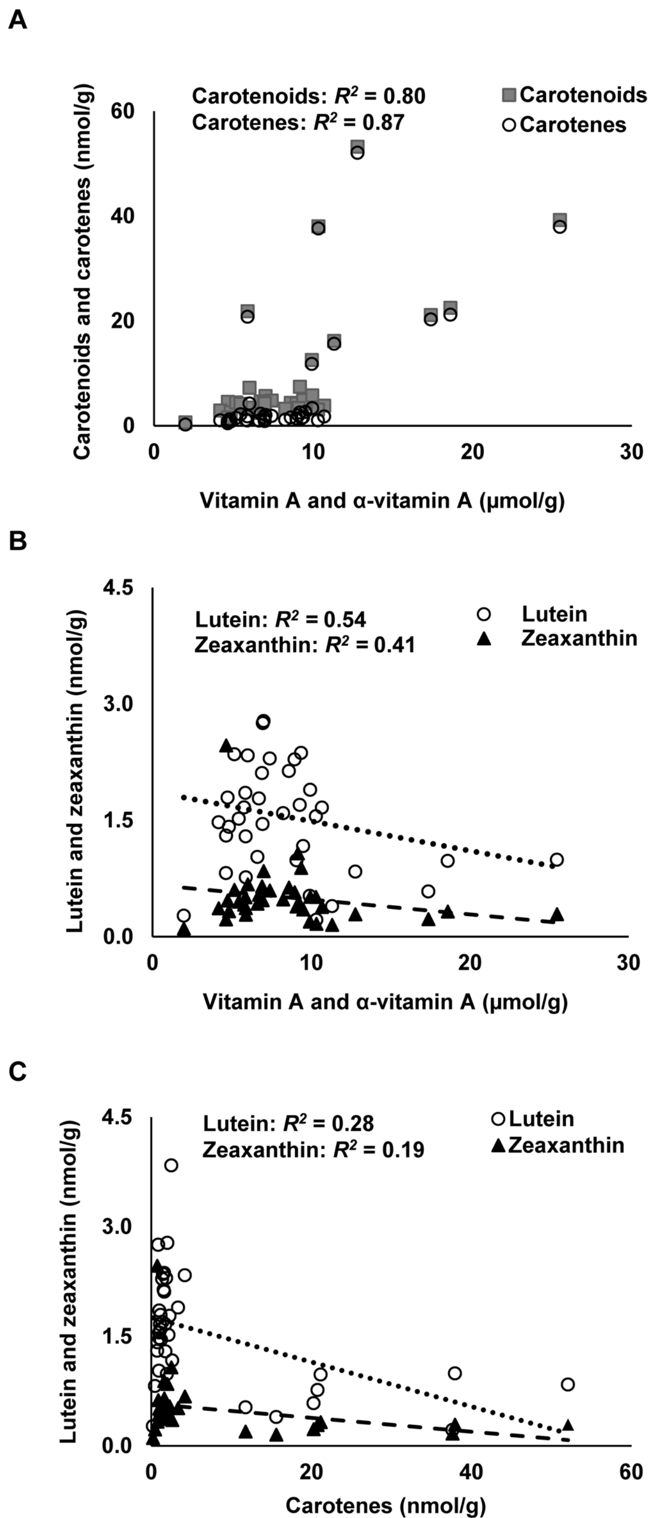

Figure 3. Hepatic carotenoid species and total vitamin A (vitamin A + α-vitamin A) concentration in adult male vervets (*Chlorocebus aethiops*; $n = 38$). The effect of diet was included in the statistical analysis. (A) Hepatic carotenoids (all identified carotenoids) and carotenes (*cis*- and *trans* α- and β-carotenes) × hepatic vitamin A and α-vitamin A concentration. (B) Lutein and zeaxanthin × hepatic vitamin A and α-vitamin A concentration. (C) Lutein and zeaxanthin × hepatic carotenes. Lutein differed by hepatic carotene content and by diet group ($P = 0.001$ for both). Squares in panel A represent carotenoids. Circles in panels B and C indicate hepatic lutein, whereas triangles represent hepatic zeaxanthin.

with hepatic liver vitamin A + α-vitamin A ($P = 0.01$; $R^2 = 0.54$; log-transformed); however, no clear pattern emerged (Figure 3 B). Hepatic zeaxanthin concentration showed no association with total hepatic vitamin A concentration (Figure 3 B). Hepatic concentrations of lutein and zeaxanthin decreased with increasing hepatic carotene concentrations (Figure 3 C).

Discussion

In this study, liver samples from adult male vervets maintained on 5 experimental diets were analyzed for vitamin A, α-vitamin A, and carotenoid concentrations. Hepatic vitamin A stores in all of the vervets in this study exceeded 1 μmol/g, the cutoff defining hypervitaminosis A in humans.[50] Furthermore, hepatic vitamin A concentrations of 4 of the 8 vervets in the OPC group exceeded 10 μmol/g, which is considered toxic in humans.[50] Hepatic vitamin A concentrations in our vervets were high (range, 1.9 to 21.4 μmol/g), but the mean was lower than previously reported for wild-caught vervets consuming laboratory feed for 2 y (14.6 ± 2.3 μmol/g)[31] and in colony-bred rhesus macaques (17.0 ± 6.3 μmol/g).[37] By comparison, human hepatic RE concentrations ranged from 0.008 to 2.86 μmol/g in 12 surgical biopsies from patients in France,[47] 0.049 to 0.56 μmol/g in 11 healthy surgical patients in the United States,[16] 0.13 to 6.31 μmol/g in 77 generally healthy men and women at autopsy,[42] and 0 to 1.97 μmol/g in 15 children and adults at autopsy.[36] Overall, hepatic α-vitamin A concentrations in our vervets ranged from 0.1 to 2.7 μmol/g liver, a generally similar range as for hepatic vitamin A concentrations in humans;[16,42,46] however, α-vitamin A concentrations were not measured in cited human studies.

When vitamin A and α-vitamin A species are considered together as an estimate of total hepatic vitamin A stores, vervets in our study had combined vitamin A stores 4 to 10 times greater than the current cutoff for hypervitaminosis A in humans (that is, 1 μmol vitamin A/g liver).[50] Although we did not analyze these samples histologically to evaluate potential hepatotoxicity and consequent adverse health effects, a study that included histologic evaluation showed hepatic stellate cell hypertrophy and hyperplasia at hepatic vitamin A concentrations of approximately 14 μmol/g in vervets.[31] Lactate dehydrogenase was elevated in half of the experimental rhesus macaques with a mean hepatic vitamin A concentration of 16.4 μmol/g,[9] likely indicating hepatotoxicity. Given the high hepatic vitamin A concentrations of vervets in the current study, liver function in at least some of our vervets likely was compromised, because hypervitaminosis A leads to hepatic injury.[39] Interestingly, although weight did not differ by diet group, vervet weight at study end was inversely associated with hepatic vitamin A concentrations, perhaps suggesting decreased food intake with increasing vitamin A intake and liver vitamin A concentration. In addition, change in weight did not differ among study groups. Anorexia and subsequent weight loss have been described in clinical hypervitaminosis A in humans,[20] and piglets dosed with 25,000, 50,000 or 200,000 IU vitamin A within 12 h of birth did not grow as well as placebo-dosed piglets.[17] Although these previous studies cannot be taken as definitive evidence for vitamin A toxicity, the potential for inadvertent vitamin A toxicity in captive NHP populations (and its unknown effects) has particular relevance for studies evaluating nutritional requirements or pharmacologic agents in NHP models.

Whereas hepatic concentrations of free retinol were not different among treatment groups, hepatic concentrations of RE, αRE, αC, βC, 9-*cis*-βC, and 13-*cis*-βC were higher but concentrations of lutein and zeaxanthin were lower in the OPC group

compared with other groups. Furthermore, vervets in the OPC group had higher hepatic vitamin A concentrations (range, 5.1 to 21.4 μmol/g) than any other treatment group, and the concentrations in the OPC vervets were similar to those in other species of NHP.[31,37] The magnitude of the difference in total hepatic vitamin A in the OPC group compared with vervets on other supplements is somewhat surprising. Although the OPC group received a large dose of provitamin A carotenoids (equivalent to approximately 12,000 IU vitamin A, according to the manufacturer's estimate of vitamin A equivalents), provitamin A conversion is regulated by dietary vitamin A in humans and Mongolian gerbils (*Meriones unguiculatus*).[4,26,27] To our knowledge, regulation of BCO1 by dietary vitamin A has not been examined specifically in vervets; however, *Chlorocebus aethiops sabaeus*,[7] along with other NHP, have an *isx* ortholog.[28,32,33] Because our vervets consumed the same HFD for several weeks prior to vitamin A treatment, differences may have been due to continued uptake of carotenoid through scavenger receptor B1, passive diffusion, or (albeit unlikely) continued species-specific BCO1-induced cleavage of provitamin A carotenoids, a phenomenon that has been observed in mice but which do not exhibit substantial regulation of BCO1 by dietary vitamin A.[48]

Differences in carotenoid absorption or provitamin A carotenoid conversion among primate species may exist. Female rhesus monkeys (n = 5) given 50 μCi ^{14}C-labeled βC in oil absorbed βC intact, albeit with high variability.[24] The majority of radioactivity was associated with the liver retinol fraction, and only approximately 2% to 8% of radioactivity was found in the liver βC fraction, suggesting that almost all of the βC was cleaved to vitamin A.[24] Chimpanzees (*Pan* spp.) and orangutans (*Pongo* spp.) circulated more lutein and RE compared with humans, regardless of dietary carotenoid profile,[18] suggesting differences even among primate species. Although not fully evaluated in vervets, a previous study observed circulation of lutein—but not RE—during a hypervitaminotic state.[31] Moreover, a cross-sectional survey of serum carotenoid concentrations at a United States zoo found substantial variation in serum carotenoids among 13 captive NHP species.[44] As expected, some of the variation probably was due to dietary preference. Sooty mangabeys (*Cercopithecus ascanius schmidti*) consumed more carotenoid than golden lion tamarins (*Leontopithecus rosalia rosalia*), which had no detectable serum carotenoid.[44] However, spider (*Ateles* spp.) and capuchin (*Cebinae* spp.) monkeys had similar dietary consumptions, but spider monkeys accumulated more serum carotenoid,[44] suggesting possible differences in carotenoid absorption or bioconversion of provitamin A carotenoids among New World primate species.

Hepatic carotenoid concentrations in the OPC group (median total carotenoid concentration, 22.2 nmol/g) were similar to concentrations observed in human biopsies.[47] Mongolian gerbils given 35 nmol αC or 17.5 nmol βC had hepatic concentrations of 1.2 nmol βC/g and 3.2 nmol αC/g, respectively.[49] In the current study, vervets had a median total hepatic βC concentration of 0.70 nmol/g liver, but that of vervets in the OPC group was 11.7 nmol/g, reflecting their increased dose. Hepatic lutein and zeaxanthin concentrations were lowest in the OPC group. Given that all vervets were fed the same HFD, this finding might suggest that supplemental carotenes compete with lutein and zeaxanthin for inclusion in micelles or for scavenger receptor B1. Evidence for such an interaction among carotenoids is available, because in healthy humans, simultaneous single-dose supplementation with βC and lutein[23] and supplementation with βC for 6 wk both decreased serum lutein.[30] High βC doses decreased serum lutein and zeaxanthin concentrations and decreased retinal zeaxanthin in chickens after 14 d.[56] This effect has not been observed consistently, because simultaneous dosing with carotenoids and lutein did not significantly affect plasma lutein AUC in healthy young men.[52]

Hepatic carotenoid concentrations were variable, with coefficients of variation ranging from 27% to 96%, depending on the carotenoid analyzed and treatment group; however, no clear pattern emerged. Other than reflecting biologic variation, this finding might be due to differences between methods or the fact that we were working with small liver samples. Vitamin A concentrations do vary throughout the liver,[36] but our recent analyses of whole human livers yielded coefficients of variation of 8% to 14% within and among liver lobes. Like vitamin A concentrations, carotenoid levels probably vary, but little is known regarding their distribution among hepatic locations. Nonetheless, hepatic concentrations of free retinol did not differ among treatment groups, and all liver RE values were high. Finally, variations in BCO1 activity due to single-nucleotide polymorphisms have been identified in chickens[22] and humans[11] and can affect plasma[27,55] and tissue response to dietary carotenoids.[22,29] Although little is known about BCO1 activity in vervets, given the existence of *bco1* polymorphisms and their effects on BCO1 activity in the human population,[27] such polymorphisms may occur in NHP as well.

In conclusion, hepatic vitamin A and α-vitamin A concentrations in these vervets were elevated, especially when the animals were supplemented with provitamin A carotenoids, and were consistent with previous reports for captive-bred NHP. Weight was negatively associated with hepatic vitamin A concentrations and may have been a result of decreased food intake. Given the effects of decreased weight with elevated liver vitamin A associated with hepatotoxicity, researchers using NHP should consider reducing the vitamin A content of research feeds. One option for vervets is to replace preformed vitamin A with βC,[5] which more closely mimics what they typically consume in nature. However, our vervets who consumed increased amounts of dietary supplemental carotenoids developed very high hepatic vitamin A concentrations. Research examining provitamin A bioconversion among NHP species is needed.

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