Twice the Amount of $\alpha$-Carotene Isolated from Carrots Is as Effective as $\beta$-Carotene in Maintaining the Vitamin A Status of Mongolian Gerbils$^1,2$

Sherry A. Tanumihardjo$^3$ and Julie A. Howe

Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, WI 53706

ABSTRACT The vitamin A (VA) value of carotenoids from fruits and vegetables is affected by many factors. This study determined the VA value of $\alpha$-carotene isolated from carrots compared with $\beta$-carotene and retinyl acetate supplements fed to Mongolian gerbils (Meriones unguiculatus). Gerbils (n = 38) were fed a VA-free diet for 4 wk. At baseline, 6 gerbils were killed to determine liver VA. Gerbils were divided into 3 treatment groups (n = 9/group) and given 35, 35, or 17.5 nmol retinyl acetate, $\alpha$-carotene or $\beta$-carotene, respectively, in 2 divided doses 5 h apart each day. The remaining 5 gerbils received oil vehicle. Gerbils were killed after 3 wk of supplementation. Serum samples and livers were collected and analyzed for VA. Liver extracts were subsequently saponified to quantify $\alpha$-retinol. Serum retinol concentrations did not differ among the groups. Liver retinyl palmitate concentrations were significantly higher in the retinyl acetate treatment group (0.198 ± 0.051 μmol/g; $P < 0.05$) than in all other groups. The $\alpha$- and $\beta$-carotene treatments resulted in similar retinyl palmitate concentrations, i.e., 0.110 ± 0.026 and 0.109 ± 0.051 μmol/g, respectively, which did not differ from the concentrations in gerbils killed at baseline (0.123 ± 0.024 μmol/g). The oil group had significantly less retinyl palmitate (0.061 ± 0.029 μmol/g; $P < 0.05$) than all other groups. $\alpha$-Retinol was detected in livers of the $\alpha$-carotene group (0.062 ± 0.013 μmol/g). Thus, twice the amount of purified $\alpha$-carotene maintained VA status as well as $\beta$-carotene in VA-depleted gerbils. Conversion factors were ~5.5 μg $\alpha$-carotene or ~2.8 μg $\beta$-carotene to 1 μg retinol.

KEY WORDS: $\bullet$ $\alpha$-retinol $\bullet$ carotenoids $\bullet$ conversion factors $\bullet$ Mongolian gerbils $\bullet$ retinol $\bullet$ vitamin A

Carotenoids currently are not considered essential nutrients for humans. A variety of phytochemicals contained in fruits and vegetables, including carotenoids, are assumed to be important for optimal health and reduced risk of chronic disease (1). In particular, high plasma concentrations of $\alpha$- and $\beta$-carotene are associated with a lower risk of atherosclerosis (2). Although health benefits are recognized, the Institute of Medicine was unable to recommend a daily intake recommendation for any carotenoid in 2000 (3). Of the provitamin A carotenoids, $\beta$-carotene, $\alpha$-carotene, and $\beta$-cryptoxanthin are the most common in the human diet. Vitamin A (VA)$^4$ equivalency factors for these carotenoids from food sources were set at 12, 24, and 24 μg, respectively, to 1 μg all-trans retinol (4). Thus, the provitamin A value of $\alpha$-carotene is assumed to be one-half that of $\beta$-carotene. This assumption is based predominately on its structure and not on scientific data. There is a paucity of studies on the VA value of $\alpha$-carotene, most likely because $\alpha$-carotene is found in nature with large amounts of $\beta$-carotene.

Carrots are the most abundant source of $\alpha$-carotene (5). Two recent studies in gerbils fed varying amounts of $\alpha$-carotene and equal amounts of $\beta$-carotene from differently colored carrots (6) showed that the $\alpha$-carotene concentration in liver increased dose dependently and did not contribute significantly to the VA pool in VA-replete gerbils. In a human study in which carrots with 2 different levels of $\alpha$-carotene were fed chronically, there was no difference in serum $\alpha$-carotene concentration during uptake and clearance (7). These studies are inconclusive concerning the VA value of $\alpha$-carotene to retinol during VA sufficiency. The carrot matrix has a negative effect on $\alpha$- and $\beta$-carotene bioavailability compared with $\beta$-carotene beadlets in ferrets (8). Moreover, evidence from the intestine of guinea pigs and rabbits suggests that $\beta$-carotene may be a more efficient substrate than $\alpha$-carotene for conversion to retinol (9).

Cleavage of $\alpha$-carotene to retinol and $\alpha$-retinol (Fig. 1) appears quantitative in weanling rats fed a VA-deficient diet (10). $\alpha$-Retinal is converted to $\alpha$-retinol during metabolism and stored in the liver, but has <2% biopotency compared with all-trans retinyl acetate (11). Absorption spectra of liver extracts from rats fed VA or $\beta$-carotene are similar, but spectra of extracts from rats fed $\alpha$-carotene show a shift in the UV maximum to 311 nm, which is the maximum for $\alpha$-retinol (10). After $\alpha$-carotene supplementation, rats had ~130% more $\alpha$-retinol than retinol based solely on inspection of the absorption spectra.

Rats are good cleavers of $\beta$-carotene and absorb very little
temperature and humidity were constant with a 12-h light:dark cycle. Gerbils were weighed daily and monitored for health until all gerbils were thriving (one gerbil died during this acclimation phase). Gerbils were dosed with 50 μL cottonseed oil daily using a 100 μL Gilson positive displacement pipette (Rainin Instruments). The VA-depletion phase lasted 4 wk.

Preparation of the doses. α-Carotene was isolated from freeze-dried high-carotene-mass carrots (Cg712–5) prepared as previously published (6). A qualitative bulk extraction of the powder was accomplished by mixing with hexane and acetone. The flask was sonicated to release the carotenoids. The solvents were decanted and the process repeated. The filtrate was concentrated using a rotary evaporator and the residue was redissolved in hexane. The hydrocarbon carotenones were enriched on a 1%-water deactivated neutral alumina column and eluted with a hexane:diethyl ether gradient, which separated α- and β-carotene from all other carotenoid pigments (5).

After enrichment, α-carotene was purified by semipreparative HPLC consisting of a 7125 Rheodyne injector; a 110B Beckman pump [4.0 mL/min; solvent, acetonitrile:methanol:dichloroethane (70:15:15, by vol; 10 mmol/L ammonium acetate)]; a Beckman Ultraphase C18, 5-μm, 10 × 250 mm column equipped with a precolumn; and an ISCO V4 absorbance detector set at 450 nm. A Spectra-Physics ChromJet Integrator recorded peaks. The first purification resulted in 93% purity. A second purification yielded α-carotene of >99% purity, α-Carotene was dissolved into cottonseed oil through sonication and the concentration determined spectrophotometrically using an E1% of 2800 at 444 nm (22); 49.2 μL oil delivered 17.5 nmol α-carotene.

α-Carotene was prepared using hexane followed by oil (6); 51 μL oil delivered 8.75 nmol β-carotene. The VA doses were prepared by directly sonicating retinyl acetate (Sigma-Aldrich) into cottonseed oil; 50 μL oil delivered 17.5 nmol VA.

Experimental design. After the 4-wk VA-depletion phase during which only oil was administered, 6 gerbils with extreme weights were killed by exsanguination while under isoflurane anesthesia; liver and blood were collected for baseline measurements. The remaining gerbils (n = 32) were assigned to 4 weight-matched treatment groups: (a) groups (n = 9) were given 35, 35, or 17.5 nmol VA, α-carotene, or β-carotene, respectively, and 5 gerbils were given 100 μL cottonseed oil. The doses were divided and one half was administered at 0800 and 1300 h each day for 3 wk. The doses chosen were physiological and based on published data estimating the basal daily VA needs of intact (12–14). α-Carotene has a negative effect on β-carotene absorption in rats (15). By feeding a mixture of α- and β-carotene to rats (1:2), hepatic retinol stores were decreased compared with β-carotene alone. When dietary α-carotene was given to rats, a conversion factor of 4.2 μg α-carotene to 1 μg of retinol was calculated from liver reserves. Considering that the VA contribution from vegetables is often questioned (16) and earlier findings in gerbils (6) and humans (7) are inconclusive, it is necessary to look specifically at the VA value of α-carotene in a model that more closely resembles humans and that permits quantification of liver reserves. Mongolian gerbils, in contrast to rats, handle α- and β-carotene more similarly to humans (6,17–21). In this study, we compare α- and β-carotene dissolved in oil administered to VA-depleted gerbils to determine bioconversion to retinol.

**MATERIALS AND METHODS**

**Gerbils and diet.** Male 41-d-old Mongolian gerbils (n = 39) were obtained from Charles River Laboratories. Gerbils were housed individually in plastic cages and given free access to food and water. All animal handling procedures were approved by University of Wisconsin-Madison’s Research Animal Resource Center. Gerbils were fed a VA- and carotenoid-free pelleted feed (Table 1). Room

![Chemical structures of retinol, α-retinol, and C23-alcohol (β-apo-carotenol). The structure of 3,4-didehydroretinol is included for comparative purposes. The chemical structures and UV-visible absorption spectra of retinol, α-retinol, and C23-alcohol (β-apo-carotenol). The structure of 3,4-didehydroretinol is included for comparative purposes.](image)

**FIGURE 1** The chemical structures and UV-visible absorption spectra of retinol, α-retinol, and C23-alcohol (β-apo-carotenol). The structure of 3,4-didehydroretinol is included for comparative purposes. 3,4-Didehydroretinol is structurally different from α-retinol, but binds to retinol-binding protein whereas α-retinol does not. α-Retinol was isolated and quantified in liver from gerbils that were administered α-carotene. C23-alcohol was used as an internal standard to determine extraction efficiency during extraction and saponification of liver esters. The compounds separated on HPLC with acetonitrile:water (87.5:12.5, v:v; 10 mmol/L ammonium acetate) at 0.7 mL/min with 2 Waters analytical columns in a series: Resolve™ C18 (5-μm, 3.9 × 300 mm) and Symmetry® C18 (3.5-μm, 4.6 × 75 mm). The chromatogram was generated at 325 nm (λ-max for retinol and intermediate between α-retinol and C23-alcohol).

![UV-visible absorption spectra of retinol (red), α-retinol (orange), and C23-alcohol (β-apo-carotenol) (green). The spectra of retinol, α-retinol, and C23-alcohol (β-apo-carotenol).](image)

![Absorbance spectra of retinol, α-retinol, and C23-alcohol (β-apo-carotenol).](image)

![Graph showing absorbance spectra of retinol, α-retinol, and C23-alcohol (β-apo-carotenol).](image)

**TABLE 1** Composition of vitamin A- and carotenoid-free diet fed to Mongolian gerbils

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Pelleted feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>463.5</td>
</tr>
<tr>
<td>Casein²</td>
<td>200.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>200.0</td>
</tr>
<tr>
<td>Cottonseed oil</td>
<td>40.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50.0</td>
</tr>
<tr>
<td>Mineral mix³</td>
<td>35.0</td>
</tr>
<tr>
<td>Vitamin mix³</td>
<td>5.0</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3.0</td>
</tr>
<tr>
<td>Cholecalcifer (500,000 IU/g), mg</td>
<td>39.7</td>
</tr>
<tr>
<td>dl-α Tocopheryl acetate (500 IU/g), mg</td>
<td>4.4</td>
</tr>
<tr>
<td>Choline dihydrogen citrate</td>
<td>3.5</td>
</tr>
</tbody>
</table>

¹ Provided by Harlan Teklad, Madison, WI.
² Vitamin-free.
³ Mineral mix (AIN-93G-MX) (36).
⁴ Vitamin mix provided the following (mg/kg feed): 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 manitol), 29.7; ascorbic acid (97.5%), 1016.6.

![Chemical structures of retinol, α-retinol, and C23-alcohol (β-apo-carotenol). The structure of 3,4-didehydroretinol is included for comparative purposes.](image)
gerbils as 3.1 μg/100 g body weight (BW) (19). After the 3-wk supplementation period, the gerbils were killed by exsanguination. Serum was prepared and livers were excised and stored at −80°C for VA and carotenoid analysis.

**Serum preparation and HPLC.** All samples were analyzed under gold fluorescent lights to prevent photooxidation and isomerization. Retinyl butyrate was synthesized and used as an internal standard (3.8 nmol/L ethanol) to determine extraction efficiency (97.7 ± 3.2%) of 500-μL standardized serum volumes, which is a modification of published procedures (6,23). The extract was reconstituted in 100 μL dichloroethane:methanol (50:50, v:v), and 50 μL was injected. The Waters HPLC system consisted of a guard column, Resolve™ C18 column (5-μm, 3.9 × 300 mm), 1525 binary HPLC pump, 717 auto sampler, and 996 photodiode array (PDA) detector. The mobile phases were acetonitrile:water (95:5, v:v; solvent A) and acetonitrile: methanol:dichloroethane (85:10:5, by vol; solvent B), both containing 10 mmol/L ammonium acetate. Samples were analyzed at 2 mL/min using a gradient procedure: 1) 100% A for 3 min, 2) 7-min linear gradient to 100% B, 3) 12-min hold, 4) 2-min reverse gradient to 100% A. Chromatograms were generated at 450 and 325 nm to quantify carotenoids and retinoids, respectively. To confirm that α-retinol was not present, increasing amounts of serum, up to 2 mL, from the α-carotene group were pooled for analysis.

**Liver retinol.** For ester analysis of the liver, 0.8–0.9 g liver was ground with ~3 g anhydrous sodium sulfate, extracted repeatedly with dichloromethane, and filtered into a 50-mL volumetric flask. Retinyl butyrate was used as an internal standard for extraction efficiency (93.7 ± 4.6%) and as an external standard. A 5-mL aliquot was dried under argon, redissolved in 100 μL methanol:dichloroethane (50:50, v:v), and 50 μL was injected. The PDA HPLC and gradient system that was used for the serum (described above) was employed for the livers.

**Liver α-carotinol.** During inspection of the liver retinyl ester elution pattern by PDA HPLC, broadening of the retinyl oleate peak was observed in the gerbils administered α-carotene. By scanning the peak, coelution with another compound was noted. By comparing this absorption spectrum (Fig. 1) with the literature (10), the compound was identified as α-retinyl palmitate. To quantify α-retinol, the livers in the α-carotene group were reanalyzed by saponifying the esters using synthesized C23-alcohol as an internal standard (Fig. 1, synthesis described below). By using C23-alcohol, the extraction efficiency could be estimated throughout the entire procedure (77 ± 8%). For reanalysis, another portion of liver (∼0.3 g) was extracted with 25 mL dichloromethane after grinding with sodium sulfate. A 5-mL aliquot was dried under argon, redissolved in 0.75 mL ethanol, and saponified with 0.4 mL potassium hydroxide:water (50:50, wt:v) at 45°C for 1 h. The reaction was quenched with 0.5 mL water and extracted 3 times with 0.5 mL hexane. The hexane layers were pooled and washed with water. The organic layer was evaporated and redissolved in 100 μL methanol:dichloroethane (50:50; v:v); 35 μL was injected. To separate α-retinol from retinol, elution was performed isocratically using acetonitrile:water (87.5:12.5; v:v; 10 mmol/L ammonium acetate) at 0.7 mL/min with a Waters Symmetry™ C18 column (3.5-μm, 4.6 × 75 mm) in series with a Waters Resolve™ C18 column (5-μm, 3.9 × 300 mm). A standard curve was constructed by purifying α-retinol from saponified liver extracts. The λ-maxima for α-retinol are 325, 311, and 298 nm (Fig. 1) and the E1%λmax is 1650 at 311 nm (10).

**Synthesis of C23-alcohol (a unique β-apo-carotenol).** C23-alcohol was synthesized using an adaptation from retinol synthesis (24). Briefly, retinal (200 mg) was dissolved in 25 mL acetone. While stirring, 1.7 mL of 1 mol/L sodium hydroxide was added dropwise. The reaction was monitored by TLC and the C23-aldehyde was extracted, concentrated, and the alcohol prepared by reduction with sodium borohydride. The reaction mixture was extracted, concentrated, and stored at −30°C. The C23-alcohol was purified as needed by isocratic HPLC. The spectrum was recorded with λ-maxima at 357 and 345 nm (Fig. 1).

**Statistical analysis and calculations.** Values are means ± SD. Data were analyzed using the General Linear Model procedure in the Statistical Analysis System (SAS Institute, Version 8.2). Outcomes of interest (i.e., gerbil weights, serum retinol concentration, liver retinol palmitate, and total liver retinol) were evaluated using ANOVA with SAS PROC MIXED, which allows the treatment variances to be different among the groups. Differences between treatment groups were determined using Least Significant Differences (LSD) at α < 0.05.

Factors for converting α- and β-carotene to retinol were calculated by comparing liver retinyl ester storage in the carotenoid-treated groups with that in the VA group. Retinol utilization rates were calculated for the oil control group compared with the baseline group and the VA group compared with the oil control group.

**RESULTS**

**Gerbil weights.** The gerbils continued to gain weight during the entire study. The final weights did not differ and ranged from 70.6 ± 4.2 g in the β-carotene group to 72.8 ± 4.7 g in the oil control group. Weights in the baseline group (61.0 ± 7.3 g) were lower (P < 0.05) because this group was killed 3 wk before the others.

**Serum and liver vitamin A and carotenoids.** Serum retinol concentrations did not differ among the groups (Table 2). The α- and β-carotene groups had the same final retinyl palmitate and total retinol liver concentrations, which did not differ from the concentrations in the group killed at baseline. Thus, 35 nmol α-carotene and 17.5 nmol β-carotene administered daily sustained the VA status of VA-depleted gerbils. The hepatic retinyl palmitate and total retinol concentrations were greater in the gerbils administered VA than in those given α- and β-carotene (P < 0.05).

No carotenoids were detected in the serum of any of the groups due to the low supplementation levels. Carotenoids were not detected in livers of the VA and oil-control groups. The β-carotene group had 3 isomers at very low concentrations. The combined β-carotene concentration was 1.2 ± 0.2 nmol/g liver (3.0 ± 0.7 nmol/liver). The α-carotene group also had 3 isomers present. The combined α-carotene concentration (i.e., 3.2 ± 0.8 nmol/g liver (8.4 ± 1.3 nmol/liver)) was higher than the β-carotene concentration in the β-carotene group because they were administered twice as much.

α-Retinol separated well from retinol after the use of a series of reverse-phase columns (Fig. 1). α-Retinol was not detected in the serum of gerbils fed α-carotene even though ample amounts were found in the liver. The liver α-retinol concentration was 0.062 ± 0.013 μmol/g, a level that com-

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>n</th>
<th>Serum retinol</th>
<th>Liver retinyl palmitate</th>
<th>Liver total retinol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline³</td>
<td>6</td>
<td>1.30 ± 0.35</td>
<td>0.123 ± 0.024b</td>
<td>0.170 ± 0.027b</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>9</td>
<td>1.44 ± 0.21</td>
<td>0.198 ± 0.051a</td>
<td>0.267 ± 0.071a</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>9</td>
<td>1.39 ± 0.09</td>
<td>0.110 ± 0.026b</td>
<td>0.155 ± 0.033b</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>9</td>
<td>1.40 ± 0.17</td>
<td>0.109 ± 0.051b</td>
<td>0.153 ± 0.066b</td>
</tr>
<tr>
<td>Oil control</td>
<td>5</td>
<td>1.57 ± 0.38</td>
<td>0.061 ± 0.029a</td>
<td>0.091 ± 0.041c</td>
</tr>
</tbody>
</table>

¹ Values are means ± SD. Means in a column with superscripts without a common letter differ, P < 0.05.
² Liver total retinol for all groups includes retinol and all identifiable esters except retinyl oleate because of substantial interference with α-retinyl palmitate in the α-carotene group.
³ Baseline group was killed 3 wk before the other groups.
pares favorably with the mean difference in total retinol concentration between the α-carotene and the oil-control groups, which was 0.064 μmol/g liver (Table 2).

Retinol conversion factors. The factors for converting α- and β-carotene to 1 μg retinol were calculated using both the difference in retinyl palmitate concentrations and the total of all esters except retinyl olate, hereafter referred to as total retinol. Total retinol does not include retinyl olate because of interference with α-retinyl palmitate in the HPLC system. The conversion factors using retinyl palmitate alone or total retinol were essentially the same. For α-carotene, 5.56 μg was equivalent to 1 μg retinol using retinyl palmitate and 5.46 μg was equivalent to 1 μg retinol using total retinol differences between groups. For β-carotene, 2.87 μg was equivalent to 1 μg retinol using retinyl palmitate and 2.84 μg was equivalent to 1 μg retinol using total retinol differences. Thus, α- and β-carotene dissolved in oil resulted in the expected ratio of VA values when twice the amount of α-carotene to β-carotene was fed.

Calculation of retinol utilization rate. The utilization rates were calculated in 2 groups of gerbils. Comparing the baseline group with the oil control group resulted in a utilization rate of 3.7 μg (13 nmol) retinol/100 g BW during the VA-depletion phase. The utilization of the VA group during the experimental treatment was calculated on the basis of the 210 μg VA this group was administered during the VA-repletion phase. Using all available liver retinyl ester data including retinyl olate for this calculation, 158.3 μg was stored in the liver of the VA-replete gerbils above that stored in the oil control group. This resulted in a utilization of 3.4 μg (12 nmol) retinol/100 g BW.

DISCUSSION

In this study, the VA value of α-carotene dissolved in oil was compared directly with β-carotene and retinol in VA-depleted gerbils. The doses were based on basal needs of 3.1 μg VA/100 g BW (19) and earlier work feeding typical carrots as the VA source to gerbils at 50 nmol/g feed (~300 nmol/d), which resulted in significant VA storage (6). We anticipated that there would be ample storage of VA in the VA group, minimal storage in the carotenoid groups, and low reserves in the oil-supplemented group. To accurately assess the conversion factor of the carotenoids dissolved in oil, an equimolar amount of VA was fed based on 100% bioconversion. The study design was such that if α-carotene yielded 1 molecule of retinol and β-carotene yielded 2 molecules, the liver reserves of the 2 groups of gerbils would be identical. Indeed, that was the result achieved.

Completely dissolving the α- and β-carotene in cottonseed oil allowed for direct comparison of the VA value. Crystalline forms of carotenoids (8,25) and the food matrices (8,26) negatively affect bioavailability. However, feeding typical carrots to gerbils increased VA stores over an equalized β-carotene supplement because carrot was fed throughout the day and the supplement was given twice daily at a much higher level than in the current study (6). Thus, carrots can lead to substantial VA storage and should be promoted in communities as a viable VA source.

This study clearly showed that twice the amount of α-carotene is as effective as β-carotene in maintaining VA stores during VA depletion. The conversion factors of ~5.5 μg α-carotene and ~2.8 μg β-carotene to 1 μg all-trans retinol in this study are slightly higher than those currently proposed by the Institute of Medicine for carotenoids dissolved in oil (4), i.e., 4 μg α-carotene and 2 μg β-carotene to 1 μg all-trans retinol. The calculated conversion rates assume that the VA utilization rates of all gerbils in this study were constant. The value of 2.8 μg β-carotene to 1 μg retinol in this study is close to the value obtained in Indonesian children using 13C-labeled β-carotene dissolved in oil, i.e., 2.6 μg β-carotene to 1 μg retinol (27). The VA status of Indonesian children is often depleted (28). Conversion rates may be dependent on VA status. The current study used a VA-depleted model; thus, VA status may partially explain the similarities in these conversion factors.

The VA utilization rate was calculated by Lee et al. (19) to be 3.1 μg/100 g BW. When the baseline liver VA was compared with that of the oil group, which was deprived of VA for 21 d longer, the VA utilization rate was 3.7 μg/100 g BW. These numbers are comparable considering that the gerbils in the current study were 3 wk younger and may have had greater requirements. The exact amount of VA given to the VA group, i.e., 210 μg, was known and provided in a highly bioavailable oil supplement at the rate of 5 μg twice daily for 21 d. The calculated VA utilization during the experimental treatment was 3.4 μg/100 g BW in the VA-replete group. The current VA recommendation is 0.72 μg/g feed (29) and gerbils consume ~6 g feed/d, resulting in 4.3 μg/d. This recommendation exceeds gerbil requirements and should result in substantial VA storage based on calculated utilization rates in ~70 g gerbils.

Serum retinol concentrations did not differ among the various treatment groups even though there were large differences in liver VA concentrations. This finding is not surprising because serum retinol concentrations are homeostatically controlled until liver reserves are dangerously low. In fact, the oil control group had a higher serum retinol concentration than the other groups even though liver reserves were significantly lower. The mean VA concentration in the control group (0.091 μmol/g) is slightly above what is considered adequate (0.07 μmol/g) for animals. Comparatively, serum retinol concentrations typically do not fall in rats until liver VA palmitate concentrations are <0.01 μmol/g (30). We attribute this slightly higher serum retinol concentration to an increased recycling of the retinol (31) because this group was not administered VA during the study duration and was certainly conserving and recirculating VA at this point in the experiment.

One interesting finding is the lack of α-retinol in serum of gerbils with ample amounts in the liver. Clearly, α-retinol does not bind to retinol-binding protein in the liver, confirming earlier in vitro studies (32). This is intriguing considering that 3,4-didehydroretinol does bind and the structural modifications are both on the ring side of the molecule (Fig. 1). Thus, α-retinol would not be a good tracer for VA status or utilization studies, but it may have utility in determining chylomicra clearance by the liver. α-Carotene is certainly converted to retinal and α-retinal in the intestinal mucosa, subsequently reduced to the alcohols and esterified to fatty acids before being packaged into the chylomicra, which circulate the esters predominantly to the liver. The fact that the α-retinol liver concentration was very similar to the retinol concentration is consistent with central cleavage of α-carotene in the intestinal brush border. α-Retinol was isolated from the liver for quantification purposes, although a synthetic process has been published (33).

α- and β-carotene were not detected in the serum of the gerbils. Other studies found nanomolar carotenoid concentrations in serum within 6 h of dosing with β-carotene and lutein (34) and when higher dietary levels of α- and β-carotene were provided (6). The utility of serum carotenoid concentration in...
gerbils for extrapolation to humans was questioned, but dose-dependent accumulation in liver was confirmed (6). The serum volume analyzed for retinol was standardized at 500 μL because systematic quantification errors were found when varying volumes were used in both serum (23) and breast milk (35). Had 1 mL serum been analyzed, carotenoids may have been above the detection limit (34), but it was deemed important to quantify retinol accurately in a standardized volume and 1 mL was not available for all gerbils. Liver storage of both α- and β-carotene was quantifiable even though the supplemental doses were low compared with carrot feeding (6).

One limitation of this study is that a group of gerbils administered α- and β-carotene was not included. However, this would have required many additional gerbils and the supply of highly purified α-carotene was limiting. For example, one would need to look at equimolar amounts of α- and β-carotene to determine absorption interference along with equimolar amounts of VA to look at bioconversion influences. Such interaction studies are better suited to the use of isotopes.

In conclusion, this study confirms that the VA conversion factor of α-carotene is twice that of β-carotene in gerbils. The carotenoids dissolved in oil are highly bioavailable and conversion rates are slightly higher than those proposed by the Institute of Medicine (4). VA utilization rates were comparable to those reported earlier (19) and support current NRC recommendations for gerbils (29). This study clearly shows that α-carotene can support VA requirements in Mongolian gerbils. Future studies determining the interaction of these 2 carotenoids should be performed. This is important because α-carotene is always found with abundant amounts of β-carotene in vegetables, but it is usually overlooked as a source of VA. This study also shows that α-retinol accumulates in liver and is not circulated in plasma, supporting earlier work suggesting that the bioactivity of α-retinol is <2% (11).

ACKNOWLEDGMENTS

The authors thank Philipp Simon, professor of Horticulture at UW-Madison, for supplying the carrots; Steven Zukerman, research assistant, for help with the gerbils; and Peter Crump, Senior Information Processing Consultant of the UW-Madison College of Agriculture and Life Sciences Statistical Consulting Service, for providing statistical assistance.

LITERATURE CITED