Ferrets (*Mustela putoius furo*) Inefficiently Convert β-Carotene to Vitamin A[1]

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ABSTRACT The ferret has recently been used as a model to evaluate the absorption and metabolism of several carotenoids; however, little is known about the vitamin A (VA) requirements of this species or the ability of ferrets to convert dietary β-carotene (βC) to VA. Three studies were conducted to estimate the daily utilization of VA in ferrets and to determine the effect of prior VA status on the ability of ferrets to utilize βC as a source of VA. Weanling male ferrets were fed a pelleted, low carotenoid, semipurified diet either with (+VA) or without VA (−VA) for 21- to 35-d prefeeding periods. Upon initiation of the experiments, several ferrets were killed to determine base-line VA status. The remaining ferrets were fed VA, βC, or VA and βC in pelleted feed (Studies 1–3) or liquid carrier (Study 3) for 16–21 additional days. Hepatic VA and βC concentrations were used as the primary indicators of VA status, although serum and adrenal VA and βC also were measured. The results showed the following: *1*) provision of βC at up to a 15:1 weight ratio of βC to VA in pelleted feed or liquid carrier was not sufficient to maintain hepatic VA stores after a −VA prefeeding period; *2*) the daily utilization rate of VA by ferrets ranged from 80 to 171 μg in the three studies; *3*) the ferret was confirmed to be a species that has the majority of its serum VA in ester form; and *4*) feeding −VA diets significantly reduced serum retinyl esters but had less effect on serum retinol. We conclude that although ferrets can convert βC to VA, the process is inefficient. The ferret model can be most appropriately used when studying the biological effect of tissue βC stores on VA status and is less appropriate for the evaluation of dietary βC conversion to VA. J. Nutr. 128: 271–279, 1998.

KEY WORDS: • *vitamin A* • *β-carotene* • *ferrets* • *bioconversion*

Vitamin A (VA)[3] deficiency is an important public health concern, particularly in developing countries where the consumption of preformed VA is low and may constitute only 10–30% of the total VA intake (FAO/WHO 1988). Increasing consumption of carotenoid-rich fruits and vegetables has been suggested as an alternative to the provision of massive oral doses of VA suggested by the World Health Organization (FAO/WHO 1986) to overcome VA deficiency. However, there is concern regarding the low efficiency with which provitamin A carotenoids are utilized from some foods (de Pee et al. 1995, Solomons 1996, Solomons and Bulux 1993). For example, de Pee and co-workers (1995) did not demonstrate improved VA status in breast-feeding Indonesian women with low VA status, who were supplemented daily with carotene-rich, dark-green, leafy vegetables, and suggested that the matrix of these foods may reduce carotenoid utilization.

Development of animal models to study the effects of complex food matrices and the effects of nutritional status of subjects and other interactions on β-carotene (βC) absorption and metabolism has been a focus in our laboratory. Ferrets, like humans, are able to absorb dietary βC intact and accumulate βC in tissue and sera. Ferrets also absorb a variety of other carotenoids such as α-carotene, lycopene and canthaxanthin (Tang et al. 1993, White et al. 1993a). In addition, ferrets convert βC to VA in the intestine (Wang et al. 1991) and in homogenates of liver, lung and adipose tissue (Wang et al. 1992). Tissue distribution of carotenoids in ferrets is similar to that of humans, with liver, adrenal and adipose tissues accumulating substantial amounts of intact carotenoids (Gugger et al. 1992, Ribaya-Mercado et al. 1989 and 1992, Zhou et al. 1996). Therefore use of the ferret as a model for studying human carotenoid metabolism seems well justified.

One difficulty with using this model is that the nutritional requirements (in particular, the VA requirement) of the ferret are not well characterized. Our laboratory has successfully developed a semipurified, pelleted ferret diet that contains 18.9 nmol/g diet (5.4 μg/g diet) VA as crystalline retinyl palmitate (a level that is within the range usually found in commercial feeds; McLain et al. 1988), which results in excellent growth (White et al. 1993b). These studies were designed to achieve the following: *1*) yield further information regarding the VA utilization rate of ferrets, *2*) determine the effects of prior VA status on the utilization of βC for VA, and *3*) compare the bioavailability of VA and βC when provided in pelleted feed or liquid carrier.

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[3] Abbreviations used: βC, β-carotene; +βC, β-carotene–containing diet; −βC, β-carotene–free diet; PDA, photodiode array; ROH, retinol, RE, retinyl esters; VA, vitamin A; +VA, vitamin A–containing diet; −VA, vitamin A–free diet.

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MATERIALS AND METHODS

Animals. Descented male ferrets (n = 47–50), 7–8 wk old with an average weight of 533, 422 and 464 g for Studies 1–3, respectively, were obtained from Marshall Farms (North Rose, NY) and certified to be of good health by a veterinarian. Animals were individually housed in stainless steel rabbit cages in the Edward R. Madigan Laboratory Animal Care Facility at the University of Illinois. Feed intake and body weight were routinely measured. The room was maintained at a constant temperature and room lighting was provided on an automated diurnal cycle of 12 h light and 12 h dark. All animal handling procedures were approved by the University of Illinois Laboratory Animal Care Advisory Committee.

Diets. The composition of the semipurified pelleted diet used was previously developed in our laboratory (White et al. 1993b), and was shown to support normal growth while depleting tissue stores of carotenoids. The pelleted diets contained either 5.41 μg/g diet VA, provided as crystalline retinyl palmitate (Sigma Chemicals, St. Louis, MO) and/or 35 μg/g (Study 1) or 65 μg/g diet βC (Studies 2 and 3) from 10% water-soluble beadlets (a gift from Hoffmann LaRoche, Nutley, NJ). This represented approximately a 6:1 and 12:1 weight ratio of βC to VA. The pelleting process used to prepare diets for the first two studies was found to be too harsh and resulted in some destruction of βC and VA. For the third study, the slurry of diet ingredients was cooled before extrusion, and during drying of pellets, less heat was used and direct light contact with pellets was avoided to reduce VA and βC degradation.

Liquid carrier preparation. *Study 3.* A liquid carrier for VA and βC was provided to two groups in Study 3. The liquid carrier was a nondairy creamer (Coffee-Mate, General Mills, Minneapolis, MN) with negligible βC or VA content. The carrier contained 25 g/100 g fat (dry basis), which should be sufficient for optimal βC absorption. The carrier was prepared daily as a 10% solution (wt/wt) of Coffee-Mate in water. β-Carotene (10% cold water–soluble beadlets) or retinyl palmitate (Palmabeads, a gift from Hoffmann-LaRoche) was added to warm Coffee-Mate, sonicated and stirred until completely dispersed. The βC from the 10% water-soluble beadlets is highly bioavailable to ferrets (White et al. 1993a).

Experimental design. *Study 1.* (**Fig. 1**A) Upon initiation of the study, four ferrets were killed by severing the brachial vessels between the pectoralis major and latissimus dorsi after cardiac puncture (described below) to determine baseline VA and βC concentrations in tissue and serum. The remaining ferrets (n = 42) were divided into groups and fed either a +VA or −VA pelleted diet for 35 d. Ferrets had free access to food and water. Seven ferrets from each group were then killed to determine the extent of VA accumulation or depletion. The remaining ferrets were fed diets containing either VA (18.9 nmol/g diet) or βC (65.2 nmol/g diet) for 16 d. Forty-eight hours before termination of the experiment, several ferrets from each treatment group were administered 6.63 Bq ^{14}C-βC (gift from Hoffmann LaRoche) orally in a 1.0 mL dose of Ensure[4] (Ross Laboratories, Columbus, OH), after overnight food deprivation. Feces were collected postdosing until termination of the experiment.

Blood samples were collected at the termination of the studies by cardiac puncture of ferrets under ketamine hydrochloride/xylazine (10:1 v/v) anesthesia (Vetallar, Parke-Davis, Morris Plains, NJ; Rompun, Miles Laboratory, Shawnee, KS, respectively) delivered by intramuscular injection (~0.30 mL/kg body weight). Tissues were removed, rinsed, blotted and weighed. Serum and tissues were stored at −20°C until analysis.

Study 2. (Fig. 1B) The design of Study 2 was similar to that of Study 1 with the following exceptions: *1)* the length of the −VA or +VA pelleted feeding was 33 d; 2) the experimental feeding was 21 d; 3) some ferrets were fed both VA and βC during the experimental period; *4)* βC concentration in the diet was 121 nmol/g diet instead of 65.2 nmol/g diet; and 5) no radioactive dose was administered.

Study 3. (Fig. 1C) Upon initiation of the study, seven ferrets were killed to determine base-line VA and βC levels in tissue and serum. Ferrets were fed a VA-deficient, semipurified, pelleted diet for 21 d at which time seven ferrets were killed to determine the extent of liver VA depletion. The remaining animals were assigned to consume one of five diets for 16 d as follows: *1)* −VA/+βC pelleted feed, *2)* −VA/−βC pelleted feed with a +βC liquid carrier, 3) +VA/−βC pelleted feed, *4)* −VA/−βC pelleted feed with a +VA liquid carrier, or *5)* −VA/−βC pelleted feed. Ferrets not receiving βC or VA in liquid carrier received 1 mL placebo carrier. Groups were adjusted to ensure similar mean body weights for all groups.

Ferrets received βC at approximately a 12:1 weight ratio of βC to VA from pelleted feed or liquid carrier. Liquid carrier treatments provided βC or VA in single, daily 1-mL oral supplement with the βC or VA content equivalent to the average quantity of βC or VA consumed the previous day by ferrets fed the corresponding βC or VA pelleted diets. The liquid carrier was administered (by 1-mL pipetman) in the early afternoon after 4–5 h of food and water

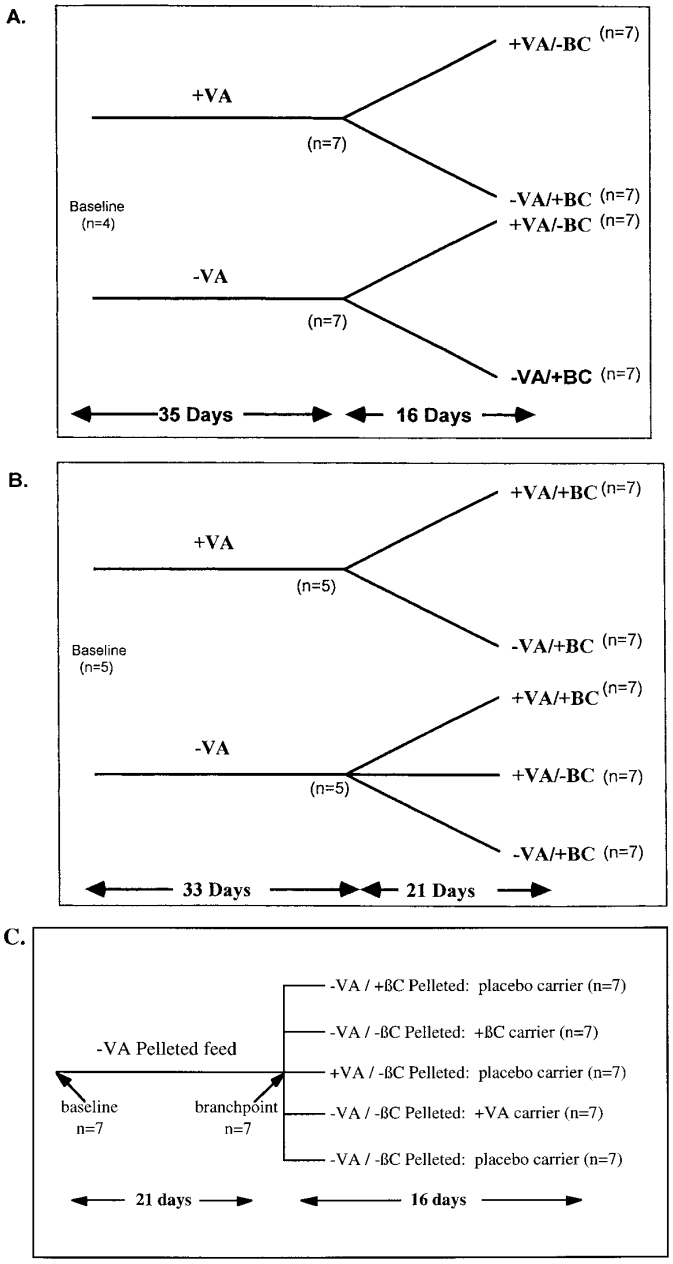

FIGURE 1 Study design for Study 1 (*A*), Study 2 (*B*) and Study 3 (*C*). All ferrets were fed a semipurified pelleted diet. The appropriate diets were supplemented with 5.41 μg/g diet vitamin A (VA) and 35 μg/g diet β-carotene (βC) for Study 1 or 5.41 μg/g diet VA and 65 μg/g diet for Studies 2 and 3.

[4] An 8-oz (240 mL) serving of Ensure contains 9 g fat, 34 g carbohydrate, 9 g protein and 131 retinol equivalents of vitamin A.

deprivation. Food and water were then returned to the ferrets. At the termination of the study, serum and tissues were removed as described previously.

Ferrets belonging to a specific diet group will be described using the notation, prefeeding diet:experimental diet.

Radiolabel dose preparation. *(Study 1).* The radiolabeled dose was prepared from purified 10, 10′, 11, 11′ ^{14}C-βC with a specific activity of 5.26 MBq/mg (a gift from Hoffmann-LaRoche). The ^{14}C-βC was dissolved in 500 μL hexane and purified by elution from a silica gel column (10-SPE, J.T. Baker, Phillipsburg, NJ) with 10 mL hexane followed by HPLC analysis. The ^{14}C-βC was added to Ensure and heated to 40°C under nitrogen to evaporate the hexane. β-Carotene beadlets (10% water-soluble beadlets, Hoffmann LaRoche) were dissolved in water and added to Ensure at a concentration of 7.45 nmol/L. The mixture was stored overnight at −20°C and warmed to 25°C before administration. The adult enteral formula Ensure was chosen on the basis of its negligible carotenoid content (Bowen et al. 1988) and ease of administration. The formula was not expected to have confounding effects on the absorption or metabolism of the βC.

Analytical methods

Diet analysis. All analysis was performed under yellow lighting. The pelleted diets were analyzed for VA and βC content before feeding. Pelleted feed was ground to a fine powder in a blender, mixed thoroughly and further ground by hand with a mortar and pestle. Triplicate samples (0.36–0.43 g) were weighed and 6 mL absolute ethanol containing BHT (1 g/L) was added. Samples were saponified by addition of 2 mL saturated KOH and incubated (70°C) in a water bath for 30 min. After cooling to room temperature, 2 mL distilled water was added and samples were extracted three times with 10 mL hexane. The combined hexane layers were completely evaporated under vacuum in a Speedvac AS160 (Savant Instruments, Farmington, NY) and the residues analyzed by HPLC for βC and VA content.

Tissue and serum VA and βC. Duplicate liver samples (0.20–0.25 g) or both adrenal glands were homogenized (Polytron 10/35, Brinkman Instrument, Westbury, NY) in 5 mL absolute ethanol containing BHT (1g/L). Samples were saponified by addition of 1 mL saturated KOH and incubated (70°C) in a water bath for 30 min. After samples were cooled to room temperature, 2 mL distilled water as added. Samples were extracted three times with 8 mL hexane and the combined hexane layers were evaporated to dryness. Echinenone (a gift from Hoffmann LaRoche) was used as internal standard for quantification of tissue βC.

β-apo-8′-Carotenic acid ethyl ester (Sigma Chemical) was added to serum as internal standard. Duplicate aliquots (0.5–1.5 mL) of serum were denatured by the addition of an equal volume of absolute ethanol containing BHT (1 g/L) and extracted three times with up to 4 mL hexane. The combined hexane layers were evaporated completely in a Speedvac and residues were analyzed by HPLC for βC and VA.

Serum retinol and retinyl-ester analysis. *(Studies 1 and 3).* Eight retinyl esters (RE) were synthesized as standards to establish HPLC retention times (all materials were purchased from Sigma Chemical). Five RE (retinyl palmitate, retinyl stearate, retinyl oleate, retinyl linoleate and retinyl myristate) were synthesized by a condensation reaction of retinol with the respective ester anhydrides in triethylamine (Hubbard et al. 1990). Arachidonate and pentadecanoate esters were synthesized by the transesterification of the methyl ester with retinyl acetate (Futterman and Andrews 1964). All standard solutions were purified on open silica columns by elution with hexane containing 50 mL/L ethyl acetate. Aliquots (0.5–2.0 mL) of serum were denatured by the addition of an equal volume of absolute ethanol containing BHT (1 g/L). Serum was extracted as described previously. Indentification of retinyl esters was based upon relative retention times to retinyl palmitate[5] and absorbance at 325 nm. Retinyl palmitate (Sigma Chemical) and all-*trans* retinol (ROH) (Sigma Chemical) standard curves were constructed and used to quantitate serum ROH and RE concentrations. Because all RE have similar extinction coefficients, the retinyl palmitate standard curve was used for all RE quantifications.

Radiolabel distribution in saponified and nonsaponified liver. *(Study 1).* For saponified liver analysis, procedures were conducted as above. Nonsaponified liver samples (0.3–0.4 g) were homogenized on ice with 3.1 mL 50 mmol/L Tris buffer. Collagenase solution (0.14 mL) [50 g/L collagenase type IV (Sigma Chemical) in PBS, pH 7.4] was added to the homogenate, vortexed and incubated at 37°C. After incubation, 0.14 mL protease solution [5 mg/mL protease type XXV (Sigma Chemical) in 50 mmol/L Tris buffer] was added and incubated at 37°C for 30 min. Samples were then cooled on ice and 3.7 mL ethanol containing BHT (1.0 g/L) was added (Peng et al. 1993). Samples were extracted three times with 8 mL hexane. Combined hexane layers were evaporated to dryness under vacuum and residues analyzed by HPLC.

For both saponified and nonsaponified liver, sample extracts were injected onto an HPLC column (no. 58298, Supelco, Bellefonte, PA) and fractions collected at 30-s intervals (Ultrorac II 2070, Pharmacia Biotech, Piscataway, NJ). The mobile phase was evaporated, and the residue was transferred to a scintillation vial containing 10 mL scintillation cocktail (Budgetsolve, Research Products International, Mt. Prospect, IL). Radioactivity of samples was obtained by liquid scintillation counting (Beckman LS 9000, Beckman Instruments, Fullerton, CA). Radioactivity eluting at times corresponding to ROH, RE and βC was verified to be associated with those compounds by a Waters 991 Photodiode Array (PDA) detector (Millipore, Bedford, MA).

Radiolabel analyses. Feces were dried at 50°C and dry weights recorded. Samples were ground with a mortar and pestle to a uniform consistency and stored at −20°C until analysis. Fecal ($n = 5$) samples were analyzed for each ferret receiving the radiolabeled dose. To prepare stock solutions, dried, ground feces (0.2 g) were solubilized with 1 mL Solvable (Dupont/NEN Research Products, Boston, MA) and incubated at 50°C in a water bath for 3 h. Samples were allowed to cool and were decolorized by the addition of 30% hydrogen peroxide. Hydrogen peroxide was added in 0.1-mL aliquots to avoid bubbling and allowed to sit overnight. Scintillation cocktail was added to aliquots of the stock fecal solution and radioactivity of the samples was obtained by liquid scintillation counting.

Serum aliquots (20 μL) were counted directly with 10 mL scintillation cocktail. Radioactivity in tissue was determined by solubilization and decolorization (when necessary), as described above, of small tissue samples (0.05–0.2 g).

Carrier analysis. *(Study 3).* Triplicate 1-mL aliquots of the βC and VA doses in liquid carrier were added to 3 mL absolute ethanol containing BHT (1g/L). Samples were saponified by the addition of 1.5 mL saturated KOH and incubated (70°C) in a water bath for 30 min. After the samples were cooled to room temperature, 2 mL of distilled water was added. The mixture was extracted four times with 6 mL hexane. The βC and VA content of the liquid carriers was determined by HPLC.

HPLC. Separate HPLC systems were used for βC and VA analyses. A Milton-Roy Constametric III Pump (Riveria Beach, FL), Rheodyne 7125 (Cotati, CA) sample injection valve, BioRad (Richmond, CA) 1790 programmable variable wavelength detector and Shimadzu (Columbia, MD) CR601 integrator were used for βC analysis. Carotenoids were separated using a Vydac C-18 (No. 201TP54) analytical column protected by an Upchurch (Oak Harbor, WA) precolumn packed with ODS C-18. The isocratic elution of carotenoids with methanol/acetonitrile/water (88:9:3, v/v/v) at a flow rate of 2 mL/min was monitored at 452 nm.

Analysis of VA was conducted on a system consisting of a Tracor (Austin, TX) pump, Rheodyne 7125 injection valve, Waters (Millipore) 486 tunable absorbance detector, and Shimadzu CR601 integrator. A Supelco LC-18 (No. 58298) column protected by an Upchurch precolumn packed with ODS C-18 was used for ROH and RE separation. Isocratic elution of ROH with methanol/acetonitrile/chloroform (47:47:6, v/v/v at a flow rate of 1.5 mL/min was detected at 325 nm. Serum RE were eluted isocratically with acetonitrile/methylene chloride (80:20, v/v with 0.5 g/L ammonium acetate added

[5] Retention times relative to retinyl palmitate: retinol (0.24), retinyl linoleate (0.71), retinyl myristate (0.80), retinyl pentadecanoate (0.88), retinyl oleate (0.94), retinyl heptadecanoate (1.06) and retinyl stearate (1.33).

TABLE 1

Tissue and serum vitamin A (VA) levels from ferrets that were fed either a VA-sufficient (+VA) or VA-deficient (−VA) diet for 35 d and then fed a diet with either VA or β-carotene (βC) for 16 d (Study 1)[1,2]

Prefeeding diet	Experimental diet	Liver VA		Adrenal VA	Serum		
					Retinol	Retinol esters[3]	Total VA[4]
		μmol/g	μmol	nmol/g	μmol/L		
Baseline	—[5]	0.60 ± 0.14[a]	17.5 ± 3.6[b,c]	32.6 ± 13.2[a,b]	1.42 ± 0.4	6.88 ± 2.6[a]	8.30 ± 3.0[a]
+VA	—[5]	0.55 ± 0.13[a,b]	25.1 ± 6.3[a,b]	41.9 ± 12.3[a]	0.94 ± 0.4	6.11 ± 2.8[a]	7.05 ± 2.8[a]
−VA	—[5]	0.17 ± 0.06[c]	7.7 ± 3.4[d]	28.3 ± 11.3[a,b]	0.96 + 0.2	3.30 ± 1.3[b]	4.25 ± 1.4[b]
+VA	+VA/−βC	0.66 ± 0.18[a]	37.8 ± 15.3[a]	45.4 ± 31.0[a]	1.32 ± 0.5	6.20 ± 1.5[a]	7.51 ± 1.7[a]
+VA	−VA/+βC	0.37 ± 0.04[b]	17.4 ± 1.9[b,c]	15.2 + 13.7[b]	1.22 + 0.5	7.58 ± 2.1[a]	8.80 ± 2.0[a]
−VA	+VA/−βC	0.23 ± 0.07[c]	12.2 ± 3.6[c]	31.1 ± 12.3[a,b]	1.32 ± 0.2	5.94 ± 2.8[a]	7.25 ± 2.9[a]
−VA	−VA/+βC	0.06 ± 0.03[d]	3.5 ± 2.5[e]	21.9 ± 11.5[b]	1.33 ± 0.5	2.08 ± 0.9[b]	3.11 ± 1.1[b]

[1] Values represent group means ± SD ($n = 7$). Values in a column with no superscripts in common are significantly different ($P < 0.05$). Liver values (μmol/g and total μmol) were log transformed before statistical analysis.
[2] See Figure 1*A* for study design.
[3] Contains the sum of retinyl esters: oleate, palmitate, stearate, linoleate, myristate, pentadecanoate and heptacanoate.
[4] Total VA is the sum of serum retinol and retinyl esters.
[5] Indicates that the ferrets in this group did not receive an experimental diet.

at a flow rate of 1.5 mL/min and detected at 325 nm (DeRuyter and DeLeenheer 1978, Furr 1990, Ross 1981).

Statistical analysis. Data were compared using one-way ANOVA and Fisher's protected least significant difference analysis (StatView 512$^+$, BrainPower, Calabasas, CA). Differences were considered significant at $P < 0.05$. Values shown represent group means ± SD. Some values were log transformed before ANOVA as indicated in table footnotes.

RESULTS

Diet analysis. Analysis of the pelleted diets used in Studies 1 and 2 revealed substantial losses (17–55%) of βC and VA. The actual concentrations of the diets were 15.7 nmol/g VA and 28.9 nmol/g βC (Study 1) and 28.9 nmol/g VA and 79.5 nmol/g βC (Study 2). Analysis of the improved, pelleted diets fed in Study 3 showed a high percentage of recovery of both βC (96%) and VA (94.5%). Similarly, analysis of the prepared oral doses showed 100% recovery of the added βC and VA. Therefore the actual weight ratio of βC to VA provided in the diets was ~3.5:1, 15:1, and 12:1 in Studies 1, 2 and 3, respectively.

Study 1

Growth. The pelleted diets provided for adequate and generally uniform growth for all groups. There were no significant differences in body weight among groups at the beginning of the experiment ($P > 0.08$). At the termination of the study, the +VA:−VA/+βC–fed group had significantly lower body weights ($P < 0.05$) than other groups, which may be explained by the slightly (but not significantly) lower initial body weight of this group. All groups grew well and did not exhibit any signs of VA deficiency (data not shown).

Tissue VA. Upon trial initiation, base-line total liver VA stores were 17.5 μmol (**Table 1**). After a 35-d prefeeding period, total liver VA stores were higher in +VA–fed ferrets and lower for ferrets fed −VA diets compared with base-line values. Ferrets fed the −VA prefeeding diet and the +VA/−βC experimental diet had significantly greater liver VA stores compared with liver stores of ferrets fed only the −VA prefeeding diet. Ferrets that were fed −VA:−VA/+βC diets had significantly lower hepatic VA stores compared with those that received only the −VA prefeeding diet. Significantly lower adrenal VA concentrations were observed in ferrets fed +VA:−VA/+βC and −VA:−VA/+βC diets compared with stores from ferrets fed only the +VA prefeeding diet or the +VA:+VA/−βC diet (Table 1).

An estimate of the daily utilization of VA was calculated by subtracting total hepatic VA stores at trial initiation from VA stores after the −VA prefeeding period and dividing by the number of days fed. The estimated utilization rate was 0.28 μmol/d (~80 μg/d).

Serum. Retinol (ROH), RE (sum of retinyl esters: oleate, palmitate, stearate, linoleate, myristate, pentadecanoate and heptadecanoate), and total VA (ROH + RE) in serum are presented in Table 1. Serum RE and total VA were both significantly lower in the −VA:−VA/+βC group and in the ferrets that were fed only the −VA prefeeding diet than all other groups. **Figure 2** shows a typical serum RE profile for ferrets fed +VA:+VA/−βC (*left panel*), showing high levels of retinyl stearate and palmitate, and ferrets fed −VA:−VA/+βC (*right panel*), showing reduced levels of retinyl stearate and palmitate.

Radiolabel analysis. Radioactivity measured in feces was highly variable, ranging from 4.1 to 63.5% recovery, and was not significantly different among treatments (data not shown). No significant differences were seen between groups in radioactivity recovered in liver and adrenal tissue; however, recovery of the ^{14}C dose from serum of the ferrets fed −VA:+VA/−βC was significantly higher than recovery form the +VA:−VA/+βC group (**Table 2**).

The distribution of radioactivity was compared between saponified and nonsaponified liver samples. The results of one ferret per treatment are shown in **Figure 3.** After saponification, radioactivity eluting with the major liver retinyl esters (palmitate and stearate) decreased as the radioactivity associated with ROH increased. Ferrets in all treatment groups converted ^{14}C-βC to VA except for those that received −VA pelleted diets during the prefeeding period followed by −VA/+βC diets in the experimental feeding period. This group had some unmetabolized ^{14}C-βC in liver (reflected by radioactivity in the peak corresponding to elution of βC + retinyl palmitate after saponification).

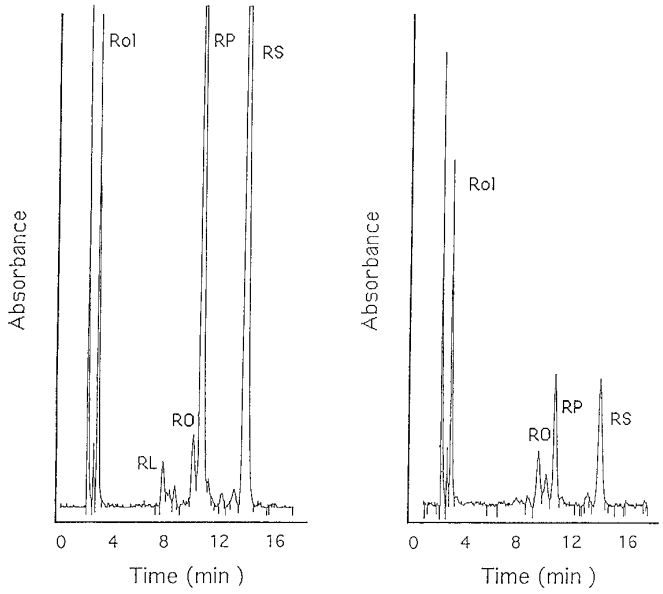

FIGURE 2 Serum retinyl ester profile of ferrets receiving either +VA:+VA/−βC (prefeeding diet:experimental diet) (*left panel*), or −VA:−VA/+βC (*right panel*). The chromatogram shows retinol (Rol) and esters: retinyl linoleate, oleate, palmitate and stearate (RL, RO, RP and RS, respectively). Diets: +VA, vitamin A–containing diet; −VA, vitamin A–free diet; +βC, β-carotene–containing diet; −βC, β-carotene–free diet.

Study 2

No differences were seen in weight gain among groups (data not shown) and no signs of vitamin A deficiency were noted. Liver total VA stores were significantly lower after the −VA prefeeding period compared with base line (**Table 3**). Ferrets fed +VA:−VA/+βC had significantly lower hepatic VA stores compared with stores of those fed only the +VA prefeeding diet. Ferrets fed either −VA:+VA/−βC or −VA:+VA/+βC had significantly higher liver VA stores compared with those fed only the −VA prefeeding diet. The concurrent provision of βC and VA resulted in significantly greater liver VA stores than did βC provided alone for both the −VA and +VA prefeed groups. The daily VA utilization rate for this study was calculated to be 105 μg.

Study 3

All groups grew well and did not exhibit any signs of VA deficiency. There were no differences in body weight among treatment groups at the onset or termination of the 16-d feeding period (data not shown). Ferrets fed liquid carrier received a daily average of 7.3 ± 0.4 μmol βC or 1.1 ± 0.2 μmol VA (12.5:1 weight ratio of βC to VA). Ferrets fed pelleted diets consumed a daily average of 7.0 ± 0.38 μmol βC or 1.1 ± 0.08 μmol VA (11.7:1 weight ratio of βC to VA).

Hepatic total VA stores of ferrets receiving +VA/−βC pelleted feed:placebo carrier or −VA/−βC pelleted feed:+VA liquid carrier were significantly higher than in ferrets after the −VA prefeeding period (branchpoint) and were significantly greater than in all other experimental groups, but were not significantly different from one another (**Table 4**). Provision of βC alone resulted in significantly lower liver VA concentrations, but not total stores, compared with values after the −VA prefeeding period. Baseline values of both liver VA concentration and total stores were significantly higher than those of any of the experimental groups.

Adrenal VA stores and concentrations were significantly higher in the experimental groups that were fed VA than in all other experimental groups (Table 4).

As expected, no βC was detected in tissues of ferrets fed diets without βC. Surprisingly, liver and adrenal βC stores were significantly greater in ferrets receiving βC from pelleted feed than in ferrets receiving βC in a liquid carrier (**Table 5**). Similarly, serum βC concentrations were significantly higher in the pelleted group than in the carrier-fed group.

Following the 21-d −VA prefeeding period, serum ROH, RE and total VA concentrations were significantly lower than base-line values (**Table 6**). Serum ROH concentrations were significantly lower in the group of ferrets fed −VA/−βC pelleted:placebo carrier than in all other groups. Serum RE concentration in ferrets fed −VA/−βC pelleted:placebo carrier was 4.4% of base-line values; however, serum ROH concentrations were 36.7% of base-line values. After the 16-d experimental feeding period, total serum VA (ROH + RE) and RE for both groups fed VA were significantly greater than all for other treatment groups, but not different from one another. Ferrets fed −VA/+βC pelleted:placebo carrier had significantly higher total serum VA and RE concentrations than ferrets fed −VA/−βC pelleted:+βC carrier.

DISCUSSION

The incidence of hypovitaminosis A in many countries around the world is quite high and leads to increased morbidity and mortality among children (Sommer et al. 1984, Sommer and West 1996). Populations in which VA deficiency is prevalent invariably rely on carotenoid-containing foods for the

TABLE 2

Radioactivity of serum, liver and adrenals from ferrets that received 6.63 Bq ^{14}C-β-carotene (βC) 2 d before termination in Study 1[1,2]

Prefeeding diet[3]	Experimental diet	Serum	Liver	Adrenal
		dpm/L	*dpm/g*	
+VA	+VA/−βC	1880 ± 410[a,b]	15,734 ± 2198	190 ± 151
+VA	−VA/+βC	1420 ± 330[b]	9009 ± 3117	144 ± 10
−VA	+VA/−βC	2640 ± 320[a]	13,670 ± 5455	250 ± 142
−VA	−VA/+βC	1930 ± 870[a,b]	12,135 ± 2454	206 ± 127

[1] See Figure 1*A* for study design.
[2] Values represent group means ± SD (n = 3). Values in a column with different superscripts are significantly different ($P < 0.05$). Adrenal data were log transformed before statistical analysis.
[3] Prefeeding diets either with (+VA) or without (−VA) were fed for 35 d and the experimental diets containing either VA or βC were fed for 16 d.

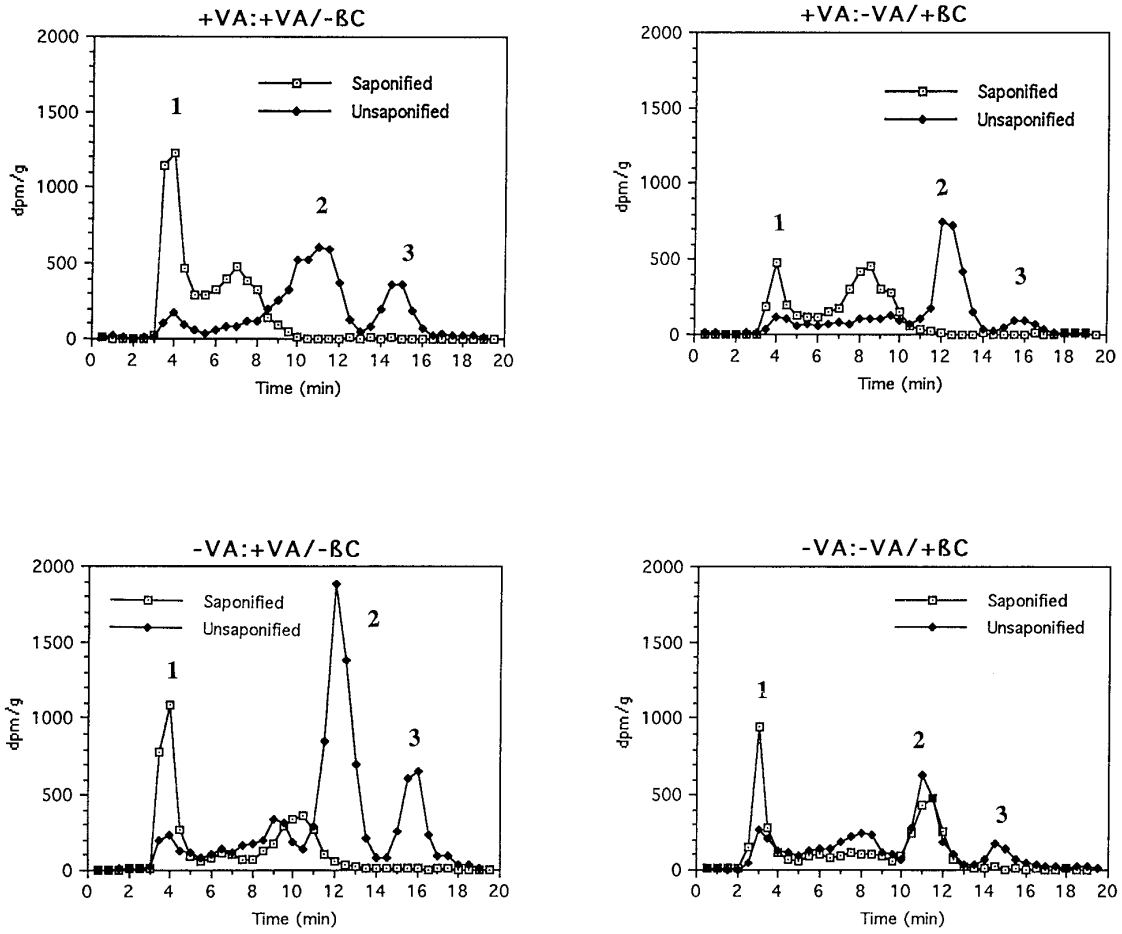

FIGURE 3 The distribution of radioactivity in saponified and nonsaponified liver tissue after a 6.63 Bq ^{14}C-β-carotene (βC) dose from one ferret per diet group in Study 1 showing that the ferrets were able to convert the βC to vitamin A (VA). Diet treatments are indicated by prefeeding diet:experimental diet. The prefeeding diet was fed for 35 d and the experimental diet for 16. The radiolabeled dose was given orally to ferrets 2 d before termination of the study. Photodiode array (PDA) analysis verified peaks 1, 2 and 3 to be retinol, retinyl palmitate +β-carotene and retinyl stearate, respectively.

majority of their VA intake (FAO/WHO 1988). Solomons and Bulux (1993) and Erdman et al. (1993) enumerated a variety of factors that contribute to the poor bioconversion of plant carotenoids to VA in humans. Among those factors are complex food matrices, intestinal parasites and low fat intakes. The VA status of the individual also may affect their ability to utilize carotenoids.

Animal models are necessary to adequately study the factors that affect the bioconversion of carotenoids from foods to VA (Van Vliet 1996). An appropriate animal model should closely mimic the human uptake, absorption and metabolism of VA and βC. The ferret model has been evaluated for uptake and tissue distribution of a variety of carotenoids (Ribaya-Mercado et al. 1989 and 1992, Tang et al. 1993 and 1995, Wang et al. 1991 and 1992, White et al. 1993a and 1993b, Zhou et al. 1996). Most notably, Wang et al. (1991) demonstrated that incubation of ferret intestinal homogenates with βC resulted in the formation of substantial quantities of retinoids and β-apo-carotenals. Moreover, perfusion of the upper portion of the small intestine with radiolabeled βC resulted in the appearance of βC and these same metabolic products in mesenteric lymph and portal circulation. A notable exception to similarities between ferrets and humans is substantially elevated serum VA in ferrets, particularly the retinyl ester contration (Ribaya-Mercado et al. 1992).

This work was designed to compare the utilization of βC and VA in pelleted diets or liquid carrier in ferrets with differing VA status. Hepatic VA stores were the major indicator of utilization. Concentrations of βC (liver, serum and adrenal), VA (serum and adrenal) and the presence of radiolabeled βC and metabolites (Study 1) also were monitored. A secondary objective was to use hepatic losses of VA in ferrets fed VA-deficient diets to estimate the daily VA utilization rate of this species. Third, the effect of VA depletion on the serum total VA and retinyl ester concentrations was studied.

Ferrets with marginal VA status were unable to utilize dietary βC at a rate sufficient to replenish needed liver VA stores. In humans, liver VA concentrations $<$70 μmol (20 μg)/g generally are considered marginally deficient, whereas concentrations $<$17.5 μmol (5 μg)/g are considered deficient (Olson 1991). Ferrets fed $-$VA diets throughout Study 1 reached liver VA concentrations as low as 6.6 μg/g yet did not convert dietary βC to VA in quantities large enough to increase or maintain liver VA stores. The actual efficiency with which ferrets convert βC to VA is not known; however, it is evident that βC provided at $\sim$ 5:1 (Study 1), $\sim$ 15:1 (Study 2) or $\sim$ 12:1 (Study 3) weight ratios was not adequate to produce hepatic VA stores equivalent to those resulting from VA feeding even though the ratios were higher than the estimated 6:1 conversion efficiency of βC to VA required to

TABLE 3

Liver Vitamin A (VA) of ferrets that were fed either a VA-sufficient (+VA) or VA-deficient (−VA) prefeeding diet for 33 d followed by an experimental diet containing VA and/or β-carotene (βC) for 21 d (Study 2)[1,2]

Prefeeding diet	Experimental diet	Liver VA	
		μmol/g	*μmol*
Baseline	—[3]	0.65 ± 0.04[a]	17.53 ± 3.07[a]
+VA	—[3]	0.34 ± 0.07[b]	16.69 ± 4.99[a]
−VA	—[3]	0.12 ± 0.03[d,e]	5.02 ± 1.79[c]
+VA	+VA/+βC	0.37 ± 0.12[b]	17.70 ± 5.05[a]
+VA	−VA/+βC	0.22 ± 0.04[c]	11.38 ± 2.16[b]
−VA	+VA/+βC	0.21 ± 0.05[c]	10.79 ± 2.53[b]
−VA	−VA/+βC	0.05 ± 0.01[e]	2.69 ± 0.61[d]
−VA	+VA/−βC	0.18 ± 0.05[c,d]	8.87 ± 2.58[b]

[1] Values represent group means ± SD, $n = 7$. Values in a column with different superscripts are significantly different ($P < 0.05$). Liver total VA stores (μmol) data were log transformed before statistical analysis.

[2] See Figure 1*B* for study design.

[3] Indicates that ferrets in that group did not receive an experimental diet.

meet the human RDA (NRC 1989) for Studies 2 and 3. In contrast, in Study 1, ferrets converted ^{14}C-βC to VA when βC was provided in the liquid carrier Ensure in a single dose. Determination of hepatic ^{14}C-βC and ^{14}C-VA metaolites indicated that all groups of ferrets efficiently converted ^{14}C-βC to ^{14}C-ROH and RE except for ferrets fed −VA/+βC diets after a −VA prefeeding period (Fig. 3), suggesting inefficient or impaired conversion with diets devoid of VA.

Neither the method of delivery (pelleted feed or liquid carrier in Study 3) nor the improvement of pelleting procedures (Study 3) improved the utilization of βC as measured by changes in hepatic total VA stores. For Study 3, liver VA concentrations did not differ in ferrets fed VA in pelleted feed or liquid carrier. Similarly, liver VA stores were the same in ferrets fed βC from either source but were significantly lower than in ferrets fed VA. Somewhat contradictory is the fact that βC concentrations in liver, adrenal and serum were greater in ferrets fed pelleted βC compared with ferrets fed βC in liquid carrier. This probably occurred because the relative percentage of bioavailibility of VA does not change over a broad range of dose levels in many species (Olson 1991), whereas the bioavailibility of βC decreases with increased dose level (Brubacher and Weisner 1985). Another explanation may be that the VA or βC from pelleted diet was fed for ~20 h/d, whereas the oral dose was provided once daily. Thus, the single daily dose of βC from the oral dose may have exceeded the ferret's absorptive capacity for βC from that "meal."

Liver VA stores for the groups of ferrets fed βC in either pelleted or liquid form from Study 3 were similar to those of ferrets that consumed the −VA/−βC pelleted diet with the placebo carrier. Similarly, Ribaya-Mercado et al. (1992) reported no difference in concentrations of liver RE and ROH in ferrets fed 80 μg of βC/g diet (compared with our 65 μg/g) for 3 wk compared with control-fed ferrets. The average intake of βC from the supplemented diets in that study was ~15.5 mg/d (compared with ~3.8 mg/d in Study 3).

Many investigators have studied the effects of VA deficiency on the utilization of dietary βC. Villard and Bates (1986) suggested that low tissue VA increased dioxygenase activity in rats. Similarly, Van Vilet et al. (1992) demonstrated increased 15,15′ dioxygenase activity in vitro in intestinal homogenates from VA-deficient hamsters. As VA status of the hamsters and rats decreased, 15,15′ dioxygenase activity increased as indicated by the increased production of VA from βC. Feeding VA depletion diets to weanling rats for 3 or 5 wk followed by oral supplementation with VA or βC in oil (emulsion-based solutions) or diet indicated that VA repletion (judged by growth rate and tissue VA accumulation) was best from the emulsion, poor from the diet and improved with severity of VA depletion (Grolier et al. 1995). Upregulation of βC cleavage has not been studied in an animal model that absorbs intact βC at physiologic doses; however, it seems logical that a similar mechanism may be involved in the conversion of βC to VA in the ferret.

Wang and co-workers (1992) demonstrated in vivo conversion of βC to VA (ROH, RE) and several β-apo-carotenoids after intestinal perfusion of ferret small intestine with a βC in micellar solution. Although these investigators did not specifically measure conversion efficiency, Wang (personal communication) estimated that the conversion of βC to VA in ferrets was ~ 12:1 under the optimal conditions of the perfusion assays. The current studies suggest that the conversion efficiency of βC to VA is < 15:1 when βC is provided in food or a liquid supplement. Thus, although this species can

TABLE 4

Liver and adrenal Vitamin A (VA) of ferrets that were fed a VA-deficient (−VA) diet for 21 d followed by a diet that contained either VA or β-carotene (βC) in either a pelleted form or a liquid carrier for 16 d (Study 3)[1,2]

Experimental diet	Liver VA		Adrenal VA	
	μmol/g	*μmol*	*nmol/g*	*nmol*
Baseline	0.64 ± 0.17[a]	14.41 ± 2.39[a]	42.87 ± 6.49[a]	2.65 ± 0.36[b]
Branchpoint[3]	0.13 ± 0.06[c]	4.86 ± 2.21[c]	16.68 ± 5.45[c]	1.65 ± .046[c,d]
−VA/−βC Pelleted:placebo carrier	0.03 ± 0.01[d]	1.41 ± 0.61[d]	7.81 ± 2.97[d]	1.29 ± 0.44[d]
−VA/−βC Pelleted:+βC Carrier	0.06 ± 0.02[d]	2.86 ± 1.14[c,d]	10.00 ± 2.50[d]	1.76 ± 0.33[c]
−VA/+βC Pelleted:placebo carrier	0.06 ± 0.02[d]	2.82 ± 0.91[c,d]	10.19 ± 3.08[d]	1.77 ± 0.54[c]
−VA/−βC Pelleted:+VA Carrier	0.26 ± 0.09[b]	11.05 ± 3.64[b]	25.43 ± 6.92[b]	4.30 ± 1.17[a]
+VA/−βC Pelleted:placebo carrier	0.24 ± 0.04[b]	10.77 ± 1.88[b]	21.02 ± 3.19[b]	3.43 ± 0.82[a,b]

[1] Values represent group means ± SD, $n = 7$. Values in a column with different superscripts are significantly different ($P < 0.05$). Adrenal total stores (nmol) data were log transformed before statistical analysis.

[2] See Figure 1*C* for study design.

[3] This group of ferrets received only the prefeeding diet and did not receive an experimental diet.

TABLE 5

Liver and adrenal β-carotene (βC) stores and serum βC concentration in ferrets fed a vitamin A (VA) deficient diet for 21 d followed by a diet containing βC in either liquid or pelleted form for 16 d (Study 3)[1,2]

Experimental diet	Liver	Adrenal	Serum
	μmol	*nmol*	*μmol/L*
−VA/+βC Pelleted:placebo carrier	1.60 ± 0.36[a]	3.22 ± 0.89[a]	3.96 ± 0.77[a]
−VA/−βC Pelleted:+βC carrier	0.25 ± 0.08[b]	0.45 ± 0.19[b]	0.48 ± 0.04[b]

[1] Values represent group means ± SD, $n = 7$. Values in a column with different superscripts are significantly different ($P < 0.05$). Serum data were log transformed before statistical analysis.
[2] See Figure 1*C* for study design.

utilize βC for VA value, the ferret is among species that are poor converters.

Our results concerning serum retinoid profile analysis support Ribaya-Mercado et al. (1992) and Tang et al. (1993) who reported high RE concentrations, in particular retinyl palmitate and retinyl stearate, in ferret serum. We found retinyl esters to be the predominant form of VA in ferret serum (51–94% of total serum VA, in Studies 1 and 3). Ferrets with the greatest liver VA stores had the highest percentage of serum RE compared with ferrets of lower VA status. Ribaya-Mercado et al. (1992) reported that serum RE represented the majority (92–94%) of the total serum VA before, after and without (control) βC supplementation of healthy, VA-sufficient ferrets. Provision of $^{14}C-\beta C$ to ferrets via an intestinal perfusion system resulted in 36 ± 6% of the absorbed $^{14}C-\beta C$ in portal vein blood associated with retinyl esters and 8 ± 2% associated with ROH (Wang et al. 1992).

High serum RE concentrations are common in carnivores (Schweigert et al. 1988); however, noncarnivores normally have < 5% of total serum VA as RE. However, elevated serum RE clearly are normal for the ferret and do not indicate a toxic VA state. With hepatic VA stores of 3.5 and 4.9 μmol, as seen in Studies 1 and 3, respectively (levels considered marginally deficient in humans; Olson, 1991), total RE still accounted for 65–67% of total serum VA. Similarly, measurable concentrations of plasma RE were observed in dogs fed VA-restricted diets for 1 y (Wilson et al. 1987). Fasting plasma RE concentrations in non-VA–supplemented, healthy humans range from 0–0.34 μmol/L (Bankson et al. 1986). Humans administered pharmacologic doses of VA had peak plasma RE concentrations of 8 μmol/L (Tang and Russell 1991). Satisfactory plasma total VA concentrations for humans range from 1.0 to 2.8 μmol/L. Clearly, ferrets have high serum RE concentrations (0.83–8.61 μmol/L in Study 3) relative to humans, indicating differences between ferret and human VA metabolism.

We conclude the following: *1*) the efficiency with which βC is converted to VA by ferrets under conditions of marginal or more deficient VA status is poorer than 15:1; *2*) the in vivo conversion of dietary βC to VA and the subsequent accumulation of VA in tissue occurred less efficiently when VA status was compromised; and *3*) the ferret has a low capacity to derive VA from βC. Thus, it appears that the ferret is not an appropriate model for evaluating the ability of carotenoids to increase VA status in VA-deficient populations. However, this species readily absorbs βC intact, and studies involving the effects of absorbed βC on metabolism should be quite appropriate.

A set of studies similar to those described above with ferrets was performed in our laboratory with the Mongolian gerbil (Lee et al. 1998), a species that absorbs physiologic doses of βC intact (Pollack et al. 1994). It was found (Lee et al. 1998) that the gerbil was much more like the human in its ability to utilize dietary βC for VA. The gerbil is thus the species of choice for βC utilization studies.

It is estimated that the daily utilization rate of VA by ferrets approaches 175 μg (estimate ranged from 80 to 171 μg in 3 studies). Feeding VA at a level of 5.41 nmol/g diet as VA resulted in appropriate growth and hepatic stores. In the two

TABLE 6

Serum retinol, retinyl esters (RE) and total vitamin A (VA) in ferrets that were fed a VA-deficient diet for 21 d followed by an experimental diet containing VA and/or β-carotene (βC) in either a liquid of pelleted form for 16 d (Study 3)[1,2]

Experimental diet	Total retinol	Total RE	Total VA
	μmol/L	*μmol/L*[3]	*μmol/L*[4]
Baseline	1.26 ± 0.29[a]	18.69 ± 2.65[a]	19.95 ± 2.81[a]
Branchpoint[5]	0.71 ± 0.12[c]	3.71 ± 1.53[c]	4.41 ± 1.59[c]
−VA/−βC Pelleted:placebo carrier	0.45 ± 0.17[d]	0.83 ± 0.31[e]	1.26 ± 0.44[e]
−VA/−βC Pelleted:+βC Carrier	0.68 ± 0.15[c]	1.62 + 0.30[d]	2.29 +0.22[d]
−VA/+βC Pelleted:placebo carrier	0.66 ± 0.11[c]	2.53 ± 0.36[c]	3.19 + 0.44[c]
−VA/−βC Pelleted:+VA Carrier	1.03 ± 0.16[b]	7.20 ± 3.06[b]	8.23 ± 3.06[b]
+VA/−βC Pelleted:placebo carrier	1.25 ± 0.20[a]	8.61 ± 2.11[b]	9.86 ± 2.02[b]

[1] Values represent group means ± SD ($n = 7$). Values in a column with different superscripts are significantly different ($P < 0.05$). All data were log transformed before statistical analysis.
[2] See Figure 1*C* for study design.
[3] Retinyl esters include the sum of retinyl: linoleate, myristate, pentadeconoate, oleate, palmitate, heptadecanoate and stearate.
[4] Total VA is the sum of total retinol and total retinyl esters.
[5] Ferrets in this group did not receive an experimental diet.

studies (1 and 3) in which retinyl esters were monitored, ferrets began with very high serum levels of retinyl esters, especially palmitate and stearate. VA depletion substantially reduced retinyl esters but had little effect on retinol concentration.

These studies provide information regarding the utilization of βC for VA stores in the ferret, an approximate utilization rate of VA for this species and data on retinyl ester changes in serum after partial depletion of VA.

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