

Waltham International Symposium: Pet Nutrition Coming of Age

The Ferret as a Model for Vitamin A Metabolism in Carnivores^{1,2}

Jens Raila,³ Claudia Gomez and Florian J. Schweigert

Institute of Nutritional Science, University Potsdam, Potsdam-Rehbrücke, Germany

EXPANDED ABSTRACT

KEY WORDS: • ferret • vitamin A metabolism • model

In contrast to the rat, the ferret (*Mustela putorius furo*) absorbs significant amounts of β -carotene from the diet and stores it in the liver and other tissues (1). Therefore, the ferret has been suggested to be an appropriate model for β -carotene metabolism in humans (2). Furthermore, recent feeding experiments have shown that dietary β -carotene and lutein may improve immunity as well as reproductive function in dogs and cats (3,4), which might be of importance when considering carotenoids as dietary supplements for these species. Ferrets share the physiological nonspecific transport of vitamin A in fasting blood plasma with canines and felids. In contrast to the human, vitamin A in plasma is present not only as retinol but also as retinyl esters (predominantly RS and RP⁴) bound to all lipoprotein fractions (5). This not only results in much higher tissue levels of vitamin A in canines but is also associated with an excretion of retinol and retinyl esters in the urine (6). Feeding trials show that this excretion is probably tightly regulated, although cellular and molecular mechanisms of this excretion are still unknown (7). To investigate whether the ferret can be used as a model to study the metabolism of vitamin A in carnivores, we conducted feeding experiments that focused on the effects of different concentrations of vitamin A in the diet on the levels of retinol and retinyl esters in plasma and organs as well as on the excretion of vitamin A in the urine.

MATERIALS AND METHODS

Animals and diets

Twelve adult (>15 mo) female ferrets (*Mustela putorius furo*), weighing 850–1250 g were obtained from the Bundesinstitut für

gesundheitlichen Verbraucherschutz und Veterinärmedizin (Berlin, Germany) and housed in the animal facility of the University. Experimental protocol and housing facilities were reviewed and approved by the animal welfare committee (Land Brandenburg, Ministerium für Landwirtschaft, Umweltschutz und Raumordnung; AZ: 48-3560-0/3). The ferrets were fed a basal diet of canned dog food (Effem GmbH, Germany), formulated to meet the nutrient requirements recommended by the National Research Council of adult dogs (8). Water was available ad libitum. The diet consisted of chicken meat, tripe and corn starch. Nutrient and energy content per kg of the basal diet was as follows: dry matter (310 g), digestible energy (5.4 MJ), crude protein (114 g), ether extracts (76 g), ash (25 g), crude fiber (6 g), calcium (4.5 g), phosphorus (4 g), sodium (2.6 g), potassium (2.4 g), magnesium (0.4 g), iron (0.16 g), copper (4 mg) and zinc (35 mg). The vitamin content per kg was 3250 IU vitamin A, 180 IU vitamin D and 100 mg vitamin E.

To assess the effect of a consecutive oral vitamin A dosing on the levels of retinol and retinyl esters in plasma, urine, liver and kidneys eight female ferrets were fed the basal diet during an experimental period of 28 d. Thereafter, one group was maintained on a basal diet (Basal) and one supplemented orally (tube feeding) with 7500 RE as RP in water (Ursovit A, Bernburg, Germany) every second d (VA+) for 37 d. At the end of the treatment period, the fasted ferrets were killed with a pentobarbital injection and samples of blood, urine, liver and kidney were collected. The effect of an oral vitamin A supplementation on the excretion of retinol and retinyl esters in the urine was studied in six female ferrets receiving an oral dose of 7500 RE as RP in water (Ursovit A) for 3 d consecutively. Urine was collected during a 12-h period each day in plastic trays beneath metabolic cages for a total of 13 d. Control samples were taken at d 0.

Analytical procedures

Plasma was separated by centrifugation (1500 \times g, 10 min, 4°C) within 4–6 h after acquisition. Urine sediment was removed by brief centrifugation (2 min) at 100 \times g. Plasma and urine were stored under nitrogen at –20°C. Tissue samples from the livers and kidneys were frozen in liquid nitrogen and kept at –80°C and analyzed within 2 mo. For separation and quantification of retinol and retinyl esters a modified gradient reversed-phase HPLC system was used (9). Retinol-binding protein (RBP) was determined in plasma and urine by Western blot. Liver and kidney sections (fixed in 4% paraformaldehyde) were stained immunohistologically using crossreacting rabbit anti-human IgG (Dako, Hamburg, Germany) (6).

¹ Presented as part of the Waltham International Symposium: Pet Nutrition Coming of Age held in Vancouver, Canada, August 6–7, 2001. This symposium and the publication of symposium proceedings were sponsored by the Waltham Centre for Pet Nutrition. Guest editors for this supplement were James G. Morris, University of California, Davis, Ivan H. Burger, consultant to Mars UK Limited, Carl L. Keen, University of California, Davis, and D'Ann Finley, University of California, Davis.

² Supported by Gesellschaft zur Förderung Kynologischer Forschung e.V., Germany.

³ To whom correspondence should be addressed.

E-mail: jraila@rz.uni-potsdam.de.

⁴ Abbreviations used: RBP, retinol-binding protein; RE, retinyl equivalent; RO, retinyl oleate; RP, retinyl palmitate; RS, retinyl stearate; VA, vitamin A.

TABLE 1

Concentrations of β -carotene, retinol, RO, RP and RS in plasma, urine, liver and kidney of ferrets treated with 7500 RE as RP for 37 d (VA+) or fed the basal diet only (Basal)¹

	<i>n</i>	β -Carotene	Retinol	Retinyl oleate	Retinyl palmitate	Retinyl stearate
$\mu\text{mol/L}$						
Plasma						
VA+	4	nd	1.53 \pm 0.97	0.07 \pm 0.01	27.54 \pm 7.37*	33.91 \pm 2.91*
Basal	4	nd	1.36 \pm 0.27	nd	4.09 \pm 1.04	5.87 \pm 0.74
Urine						
VA+	4	nd	7.60 \pm 2.16*	0.10 \pm 0.10	0.05 \pm 0.03	43.06 \pm 21.46
Basal	4	nd	0.90 \pm 0.24	nd	nd	n
$\mu\text{mol/g wet tissue}$						
Liver						
VA+	4	nd	64.66 \pm 46.83	474.8 \pm 272.2*	851.5 \pm 349.0*	15.21 \pm 10.40*
Basal	4	nd	25.12 \pm 16.26	51.74 \pm 7.42	232.4 \pm 209.5	2.18 \pm 2.80
Kidney						
VA+	4	nd	13.22 \pm 2.61	1.52 \pm 1.28	4.40 \pm 3.88	0.09 \pm 0.01
Basal	4	nd	7.92 \pm 4.64	0.45 \pm 0.32	2.13 \pm 0.34	0.09 \pm 0.01

¹ Data are expressed as means \pm SD.

* Significantly different from the Basal group ($P < 0.01$ by Student's *t*-test). nd, below detection limit.

Statistical analysis

The data are expressed as the mean and standard deviation (SD). To compare values, analysis of variance (ANOVA) and Student's *t*-test in the SAS software (SAS Institute, Cary, NC) were used. The probability level at which differences were considered significant was $P < 0.05$.

RESULTS

The dietary supplementation of vitamin A (Table 1) resulted in sixfold higher plasma levels of retinyl esters ($P < 0.01$) but had no effect on plasma retinol. The dominant ester was RS (57.00 \pm 6.65%) followed by RP (42.86 \pm 6.68%) and RO (0.14 \pm 0.04%). Western blot analysis revealed a 21-kDa protein corresponding to RBP. No differences in RBP between the supplemented and unsupplemented ferrets were observed (Fig. 1). The supplementation of vitamin A (Table 1) resulted in significantly higher concentrations of total vitamin A (primarily RP) in the liver ($P < 0.05$) but not in the kidneys ($P = 0.07$). Four wk after vitamin A supplementation, the retinol and retinyl ester concentrations

in urine were significantly ($P < 0.01$) higher in the supplemented group compared to that of the unsupplemented group. Figure 2 shows the transient increase from 7.26 \pm 5.44 to 763 \pm 314 nmol/L in RP levels ($P < 0.01$) in urine of ferrets after an oral dose of 7500 RE as RP on 3 d consecutively. In ferret livers, specific cytoplasmic staining for RBP was observed in all parenchymal cells (hepatocytes). Periportal intensity was stronger than centrilobular intensity (Fig. 3). No obvious differences were observed in the intensity of immunoreactivity between the two feeding groups (VA+, Basal). In the kidneys, immunoreactive RBP was observed exclusively in the epithelial cells of the proximal convoluted tubules of the renal cortex (Fig. 4).

DISCUSSION

As in canines and other carnivores, ferrets have high concentrations of retinyl esters in plasma. In contrast to tissues, the plasma retinyl esters are primarily present as RS (5,10). Similarly to dogs (7) plasma levels of retinyl esters, but not of retinol, are affected by the dietary intake of vitamin A. This

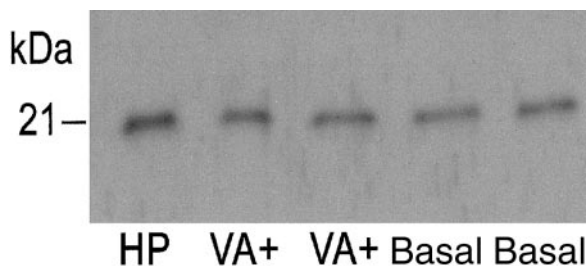


FIGURE 1 Immunoblot analysis of retinol-binding protein (RBP) in human and ferret plasma. Five micrograms of total protein were subjected to SDS-PAGE, transferred to PVDF membranes and incubated with crossreacting rabbit anti-human RBP IgG (1.3 $\mu\text{g/mL}$). Bound IgG was visualized using the chemiluminescence reaction. No differences between the supplemented (VA+) and unsupplemented (Basal) ferrets were observed. HP, human control plasma.

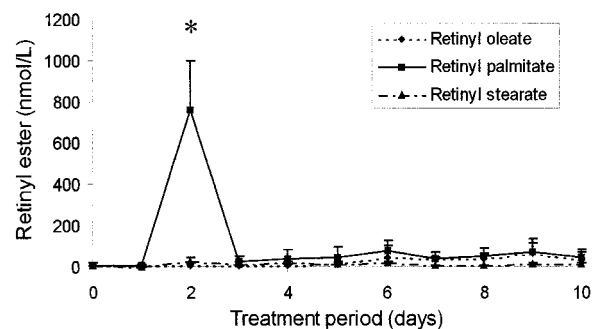


FIGURE 2 Concentrations of RO, RP and RS in urine from female ferrets given daily oral doses of 7500 RE as RP at d 1, 2 and 3 (d 0 = before the first dose). Values are means \pm SD *Significantly different from baseline values ($t = 0$ by ANOVA and Dunnett's multiple comparison test), $P < 0.05$.

can be explained by the fact that in dogs and ferrets retinyl esters are transported by lipoproteins (11), whereas retinol is associated with its specific binding protein, the RBP, which has been demonstrated immunologically in plasma of dogs (6) and for the first time in ferrets. Immunohistological investigations performed in this study support the hypothesis that the liver parenchymal cells are the primary site of RBP synthesis and secretion into the blood (12). In the kidneys, the tubular reabsorption prevents urinary loss of RBP-retinol under physiological conditions by a megalin-mediated endocytosis (13). The RBP labeling observed in the proximal tubules as well as the observation that no RBP was present in the urine indicate that ferrets take up RBP-retinol by receptor-mediated endocytosis from the tubular fluid as has been suggested in other canine species (6). The results further show that ferrets have the capacity to excrete retinol and retinyl esters in the urine. The response in the urine seems to be dependent on the oral vitamin A supplementation, which is to some extent in accordance with results for vitamin A from a long-time feeding experiment in healthy dogs (7). The excretion of vitamin A in the urine of ferrets indicates that the kidney possesses a specific role in vitamin A metabolism. Although RBP-associated retinol is efficiently reabsorbed in the proximal tubules, both retinyl esters and retinol are excreted.

In conclusion, feeding experiments performed in this study show that ferrets and dogs behave in a similar manner with regard to vitamin A metabolism. Therefore the ferret can be used as a model to investigate aspects of β -carotene metabolism as well as aspects of the metabolism of vitamin A such as absorption in the gut, regulation of incorporation of retinyl esters into lipoproteins in the liver as well as the renal uptake and regulated excretion of vitamin A in the urine. However, the substantial differences in vitamin A metabolism of ferrets compared to that of most other mammals and humans have to be considered, if the ferret is used as a model for β -carotene metabolism in humans.

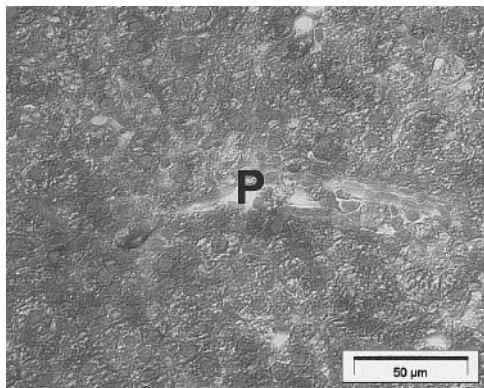


FIGURE 3 Localization of immunoreactive RBP in ferret liver sections using rabbit anti-human RBP IgG (1:400). Bound IgG was visualized by diaminobenzidine using the peroxidase anti-peroxidase method. A strong cytoplasmic staining of parenchymal cells is seen in the periportal parenchymal cells. P, portal triad. (Immunohistology, Normarski interference contrast.)

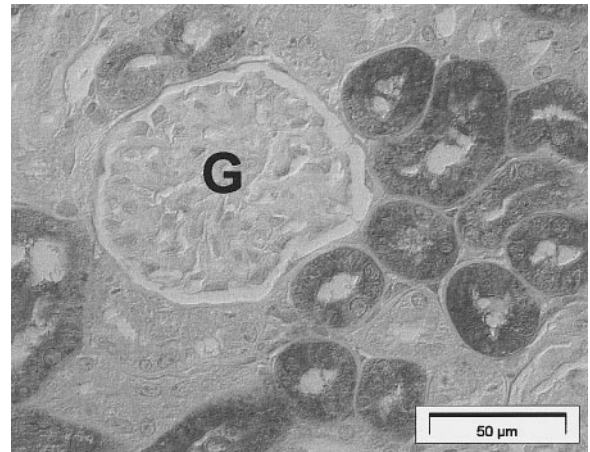


FIGURE 4 Localization of RBP using rabbit anti-RBP IgG (1:400) on ferret kidney sections. Bound IgG was visualized by diaminobenzidine using the peroxidase anti-peroxidase method. Strong labeling for RBP is seen in the cells lining the proximal convoluted tubules. G, glomerulus. (Immunohistology, Normarski interference contrast.)

LITERATURE CITED

- Ribaya-Mercado, J. D., Fox, J. G., Rosenblad, W. D., Blanco, M. C. & Russell, R. M. (1992) Beta-carotene, retinol and retinyl ester concentrations in serum and selected tissues of ferrets fed beta-carotene. *J. Nutr.* 122: 1898–1903.
- Lee, C. M., Boileau, A. C., Boileau, T. W., Williams, A. W., Swanson, K. S., Heintz, K. A. & Erdman, J. W., Jr. (1999) Review of animal models in carotenoid research. *J. Nutr.* 129: 2271–2277.
- Kim, H. W., Chew, B. P., Wong, T. S., Park, J. S., Weng, B. B., Byrne, K. M., Hayek, M. G. & Reinhart, G. A. (2000) Modulation of humoral and cell-mediated immune responses by dietary lutein in cats. *Vet. Immunol. Immunopathol.* 73: 331–341.
- Weng, B. C., Chew, B. P., Wong, T. S., Park, J. S., Kim, H. W. & Lepine, A. J. (2000) Beta-carotene uptake and changes in ovarian steroids and uterine proteins during the estrous cycle in the canine. *J. Anim. Sci.* 78: 1284–1290.
- Ribaya-Mercado, J. D., Blanco, M. C., Fox, J. G. & Russell, R. M. (1994) High concentrations of vitamin A esters circulate primarily as retinyl stearate and are stored primarily as retinyl palmitate in ferret tissues. *J. Am. Coll. Nutr.* 13: 83–86.
- Raila, J., Buchholz, I., Aupperle, H., Raila, G., Schoon, H. A. & Schweigert, F. J. (2000) The distribution of vitamin A and retinol-binding protein in the blood plasma, urine, liver and kidneys of carnivores. *Vet. Res.* 31: 541–551.
- Schweigert, F. J. & Bok, V. (2000) Vitamin A in blood plasma and urine of dogs is affected by the dietary level of vitamin A. *Int. J. Vitam. Nutr. Res.* 70: 84–91.
- NRC. (1985) *Nutrient Requirements of Domestic Animals: Nutrient Requirements of Dogs.* National Academy of Sciences, National Research Council, Washington, DC.
- Schweigert, F. J., Hurtienne, A. & Bathe, K. (2000) Improved extraction procedure for carotenoids from human milk. *Int. J. Vitam. Nutr. Res.* 70: 79–83.
- Schweigert, F. J., Ryder, O. A., Rambeck, W. A. & Zucker, H. (1990) The majority of vitamin A is transported as retinyl esters in the blood of most carnivores. *Comp. Biochem. Physiol. A* 95: 573–578.
- Ribaya-Mercado, J. D., Lopez-Miranda, J., Ordovas, J. M., Blanco, M. C., Fox, J. G. & Russell, R. M. (1993) Distribution of beta-carotene and vitamin A in lipoprotein fractions of ferret serum. Effect of beta-carotene supplementation. *Ann. N.Y. Acad. Sci.* 691: 232–237.
- Blomhoff, R., Green, M. H., Green, J. B., Berg, T. & Norum, K. R. (1991) Vitamin A metabolism: new perspectives on absorption, transport, and storage. *Physiol. Rev.* 71: 951–990.
- Christensen, E. I., Moskaug, J. O., Vorum, H., Jacobsen, C., Gundersen, T. E., Nykjaer, A., Blomhoff, R., Willnow, T. E. & Moestrup, S. K. (1999) Evidence for an essential role of megalin in transepithelial transport of retinol. *J. Am. Soc. Nephrol.* 10: 685–695.