Journal of Veterinary Medicine Series A

Zentralblatt für Veterinärmedizin Reihe A

Physiology, Pathology, Clinical Medicine

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VOLUME/BAND 38, 1991

302 Figures / Abbildungen
189 Tables / Tabellen

BIBLIOTHEK
der Tierärztlichen Fakultät
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8000 München 22
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PAUL PAREY Scientific Publishers Berlin and Hamburg

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J. Vet. Med. A 38, 35–42 (1991)
© 1991 Paul Parey Scientific Publishers, Berlin and Hamburg
ISSN 0931–184X

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Vitamin A (Retinol and Retinyl Esters), α-Tocopherol and Lipid Levels in Plasma of Captive Wild Mammals and Birds[1]

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With 2 tables

(Received for publication August 10, 1990)

Summary

Vitamin A (retinol and retinyl esters), vitamin E and lipids were determined in a wide variety of wild mammals and birds held in captivity. In mammals plasma levels of vitamin A were generally below 500 ng/ml and those of vitamin E were highly variable (0.1–2 µg/ml). In primates, vitamin E levels were 3 to 8 µg/ml. Whereas in Marsupalia, Chioptera, primates, Rodentia, Proboscidea, Sirenia, Perissodactyla and Artiodactyla only retinol was found, retinyl esters (basically retinol palmitate/oleate) represented 10 to 50 % of the total plasma vitamin A in some birds of the order Ciconiiformes and Falconiformes. Retinol levels in birds were higher compared to mammals (500–2,000 ng/ml). The same was true for lipids as well as for vitamin E levels (1–26 µg/ml) in the plasma of birds.

Introduction

Recent studies on vitamin A transport in blood plasma showed that there are distinct differences within the animal kingdom. Especially in the order of carnivores, a nonspecific transport of vitamin A esters by lipoproteins seems to be physiological and not associated with any signs of vitamin A intoxication (Schweigert, 1988; Schweigert et al., 1990). These novel observations raise the question as to whether this phenomenon is unique for the order Carnivora or whether other species show similar peculiarities in vitamin A transport. Although vitamin A levels of numerous species are known, difficulties in their interpretation arise from the methods of determination used. Based on the knowledge that vitamin A is transported under physiological conditions nearly exclusively as retinol (> 95 % of total vitamin A), most of the methods used so far have only considered the alcoholic form of vitamin A. In carnivores, for example, this would yield values which would be 60–99 % too low. In this paper, therefore, vitamin A (retinol and retinyl esters),

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[1] This paper is dedicated to Professor Dr. Michael Merkenschlager on the occasion of his 65th birthday.

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vitamin E and lipids were determined in a wide variety of wild mammals and birds held in captivity. Additionally, numerous diseases associated with vitamin A or E deficiency are known in wild animals held in captivity (KONSTANTINOV and IPPEN, 1979; ZWART et al., 1979; KOČI, 1982; LIU et al., 1983; LIU and DOLENSEK, 1986; for a review on vitamin E see DIERENFELD, 1989). Their evaluation would benefit from the knowledge of normal vitamin A and E plasma values, which are rather rare in literature.

Material and Methods

Plasma or serum samples were obtained from wild animals kept in captivity under various housing and feeding conditions at the zoological gardens in München (Tierpark Hellabrunn, ZH), the zoological gardens in Nürnberg (ZN), the Institut für Geflügelkrankheiten (Veterinary Faculty) (GK), the Pettenkofer Institute (PI), a falconry (Jagdfalkenhof Schloß Rosenburg) (F) and the local slaughterhouse. Samples were frozen between −20° and −70 °C and stored until further analyzed. Lyophilized serum of the rattle snake (pooled) was obtained from Sigma (St. Louis, MO, USA).

Vitamins A and E were determined with an isocratic reversed-phase high-performance liquid chromatography (HPLC) system as described elsewhere (SCHWEIGERT, 1990). Briefly, vitamins A and E were extracted from the serum or plasma and separated on a RP-18 column (4 × 125 mm, 5 µm) using methanol as the eluent at a flow rate of 1 ml/min. Vitamin A was detected at 313 nm and a fluorescence spectrophotometer was used to quantify vitamin E (α-tocopherol). Wavelength settings were 295 and 330 nm for excitation and emission, respectively. β-Carotene was separated on straight-phase HPLC as described (VUILLEUMIER et al., 1983). Retinol, retinol palmitate, retinol stearate, α-tocopherol and β-carotene were identified and quantified by comparison of retention time as well as peak areas with external standards. Recovery was more than 93 % for all components. The detection limit for retinol, retinyl esters and α-tocopherol was 2 ng, 3.5 ng and 2.4 ng, respectively and 2 ng for β-carotene. Cholesterol, triglycerides and phospholipids were measured by enzymatic methods using commercial assay reagents (Boehringer Mannheim, FRG).

Results

Table 1 summarizes the results for retinol, α-tocopherol and lipid in the plasma of different species in the order of Marsupalia, Chioptera, primates, Rodentia, Proboscidea, Sirenia, Perissodactyla and Artiodactyla. Retinyl esters were only detectable in traces in Marsupalia and in primates, especially in animals of the species tamarin (71 ± 94 ng/ml, retinol equivalent). β-Carotene was only present in the plasma of animals found in the orders Perissodactyla (wild horse, 377 ± 594 ng/ml; kulan, traces; domestic donkey, 183 ng/ml; mountain zebra, traces) and Artiodactyla (cow, 1,021 ± 880 ng/ml; gaur, 1,826 ng/ml). Retinol levels in the plasma were generally lower than 500 ng/ml. In bats only traces of retinol could be detected. Contrary to this, levels of α-tocopherol were highly variable. In nearly all plasma samples of the investigated species, phospholipids represent the majority of lipids followed by cholesterol and triglycerides. In birds (Table 2) not only retinol but in some species of the order Ciconiiformes and Falconiformes, retinyl esters were detected as well. The dominant ester fraction was retinyl palmitate/oleate. Samples of birds were not investigated for β-carotene. Both α-tocopherol and lipids were much higher in birds compared to mammals. The only reptile investigated — a rattle snake *(Crotalus spp.)* — had a low level of retinol (23 ng/ml). α-Tocopherol, phospholipids and triglycerides were found to be 8.44 µg/ml, 1.28 and 1.62 mg/ml, respectively.

Discussion

In the present study almost 250 serum or plasma samples from a fairly wide range of wild mammals and birds (59 species) held in captivity were investigated for their levels of fat-soluble vitamins A and E as well as lipids. Despite this large number of samples, differences in age, sex, nutritional status and environment as well as sampling and sample handling make comparison difficult not only within the material of this study, but also with results reported by others. The only variables in this study which are known to show very little effect on the concentration of fat-soluble vitamins are the kind of sample (plasma vs. serum) as well as sample storage and transportation (CRAFT et al., 1988).

Table 1. Vitamin A, α-tocopherol and lipid levels in plasma of mammals

Species	n	Retinol ng/ml	α-Tocopherol μg/ml	Phospholipids	Cholesterol mg/ml	Triglycerides
Marsupalia (ZH)						
Red kangaroo *(Macropus rufus)*	6	146 ± 44	1.81 ± 0.35	1.56 ± 0.26	0.92 ± 0.20	0.76 ± 0.15
Wallaroo *(Macropus robustus)*	2	134 / 109	0.27 / 0.26	1.61 / 1.59	0.92 / 0.92	1.19 / 0.52
Doria's tree kangaroo *(Dendroagus dorianus)*	1	197	1.02	2.04	1.10	0.33
Pretty-face wallaby *(Protemnodon elegans)*	3	157 ± 37	2.52 ± 2.09	2.32 ± 0.76	1.37 ± 0.37	0.73 ± 0.29
White-throated wallaby *(Protemnodon parma)*	1	126	0.18	1.13	0.72	0.81
Common opossum *(Didelphis marsupialis)* (S)	1	21	1.48	1.71	1.48	0.33
Chioptera (ZI)						
Naked-backed bat *(Pteronotus pannellii)*	1	traces	0.16	0.39	0.31	0.10
Horseshoe bat *(Rhinolophus rouxi)*	5	traces	0.25 ± 0.28	0.40 ± 0.32	0.14 ± 0.09	0.34 ± 0.18
Primates						
Siamang *(Hylobates syndactylus)* (ZH)	2	440 / 445	8.02 / 5.84	nd	nd	nd
Common marmoset *(Callithrix jacchus)* (PI)	15	125 ± 79	6.00 ± 10.35	nd	nd	nd
Brown-headed tamarin *(Saguinus fuscicollis)* (PI)	17	90 ± 63	3.30 ± 2.47	nd	nd	nd
Rodentia						
Rat *(Rattus rattus)*	6	188 ± 36	6.17 ± 2.10	1.62 ± 0.31	0.95 ± 0.10	1.09 ± 0.42
Proboscidea (ZH)						
African elephant *(Loxodonta africana)*	1	38	0.12	0.90	0.55	0.59
Indian elephant *(Elephas maximus)*	1	45	0.06	0.44	1.04	0.46
Sirenia						
Manatee *(Trichechus manatus)* (ZN)	1	35	1.60	2.23	1.92	0.75
Perissodactyla (ZH)						
Horse *(Equus caballus)*	14	213 ± 60	1.30 ± 0.54	nd	0.78 ± 0.13	nd
Wild horse *(Equus przewalskii)*	6	163 ± 43	1.21 ± 0.35	1.11 ± 0.48	0.68 ± 0.37	0.32 ± 0.16
Kiang *(Equus hemionus kiang)*	5	101 ± 17	1.64 ± 0.73	0.56 ± 0.12	0.58 ± 0.15	0.09 ± 0.04
Kulan *(Equus hemionus kulan)*	8	139 ± 41	0.78 ± 0.30	0.70 ± 0.32	0.81 ± 0.40	0.26 ± 0.16
Domestic donkey *(Equus asinus asinus)*	1	179	2.00	1.66	0.92	0.73

Somalian wild ass *(Equus asinus somalicus)*	1	37	1.03	1.22	0.64	0.30
Mountain zebra *(Equus zebra hartmannae)*	1	88	1.85	0.39	0.52	0.13
Artiodactyla (ZH)						
Domestic pig *(Sus scrofa domesticus)*	10	174 ± 54	1.51 ± 0.36	1.07 ± 0.15	0.92 ± 0.09	0.35 ± 0.17
Vicugna *(Vicugna vicugna)*	1	308	0.32	0.36	0.29	0.24
Fallow deer *(Dama dama dama)*	1	158	0.98	1.28	0.79	0.15
Barasingha *(Cervus duvauceli)*	1	249	0.21	0.70	0.55	0.29
Red deer *(Cervus elaphus)*	2	279 / 233	0.76 / 0.65	0.50 / 0.48	0.37 / 0.36	0.08 / 0.05
Père David's deer *(Elaphurus davidianus)*	2	132 / 114	1.71 / 1.32	0.78 / 0.60	0.58 / 0.45	0.18 / 0.12
Moose *(Alces alces)*	4	297 ± 21	0.95 ± 0.26	0.83 ± 0.40	0.50 ± 0.22	0.21 ± 0.06
Nilgai *(Boselaphus tragocamelus)*	1	279	0.96	0.74	0.45	0.08
Domestic ox *(Bos taurus)*	13	308 ± 53	2.30 ± 0.74	1.57 ± 0.27	1.56 ± 0.32	0.14 ± 0.04
Gaur *(Bos gaurus)*	1	40	0.60	0.52	0.77	0.17
Blesbok *(Damaliscus dorcas philippsi)*	1	428	0.49	0.37	0.27	0.07
Dama gazelle *(Gazella dama mhorr)*	12	489 ± 98	0.89 ± 0.52	0.48 ± 0.16	0.44 ± 0.14	0.28 ± 0.78
Chamois *(Rupicapra rupicapra)*	3	1939 ± 2915	0.79 ± 0.40	0.78 ± 0.34	0.46 ± 0.20	0.22 ± 0.24
Ibex *(Capra ibex)*	3	815 ± 36	0.67 ± 0.30	0.71 ± 0.13	0.50 ± 0.20	0.32 ± 0.66
Markhor *(Capra falconeri)*	11	435 ± 108	1.58 ± 1.16	0.89 ± 0.31	0.73 ± 0.27	0.44 ± 0.38
Wild goat *(Capra aegagrus hircus)*	1	297	0.26	1.12	0.69	0.47
Mouflon *(Ovis ammon musimon)*	1	260	0.10	0.46	0.29	0.65
Domestic sheep *(Ovis ammon aries)*	1	302	0.57	0.78	0.51	0.35

Code: ZH = Zoological garden Hellabrunn — München; ZN = zoological garden Nürnberg; PI = Pettenkofer Institut; S = Sigma Chemicals. nd = not determined. The results can be converted into SI units by using the following factors: retinol 0.0035 (ng/ml into µmol/l); α-tocopherol 2.32 (µg/ml into µmol/l); phospholipids 1.29; cholesterol 2.59; triglyceride 1.15 (all mg/ml into mmol/l).

Table 2. Vitamin A, α-tocopherol and lipid levels in plasma of birds

Species	n	Retinol	*R. palmitate/ oleate*	*R. stearate*	α-Tocopherol	Phospholipids	Cholesterol	Triglycerides
			ng/ml		µg/ml		mg/ml	
Pelecaniformes (ZH)								
Eastern white pelican *(Pelecanus onocrotalus)*	1	692	–	–	8.42	3.06	1.55	1.00
Dalmatian pelican *(Pelecanus crispus)*	1	580	–	–	15.69	3.40	1.36	0.87
Ciconiiformes (ZN)								
White stork *(Ciconia ciconia)*	1	245	103	–	2.98	2.83	1.60	0.57
Black stork *(Ciconia nigra)*	1	768	144	–	9.00	1.95	1.52	1.04
Hermit ibis *(Geronticus eremita)*	3	502 ± 11	–	–	7.86 ± 0.96	2.77 ± 0.39	1.75 ± 0.28	0.44 ± 0.03
Falconiformes (GK)								
Sparrowhawk *(Accipiter nisus)*	2	1664 / 1129	276/–	87/–	24.87 / 24.17	4.69 / 3.41	3.45 / 2.24	4.13 / 0.59
Goshawk *(Accipiter gentilis)*	2	1824 / 1144	683/traces	210/–	25.96 / 7.32	3.66 / 2.07	3.38 / 2.29	0.94 / 0.65
Common buzzard *(Buteo buteo)* (F)	1	804	110	–	22.77	nd	4.07	1.36
Bonelli's eagle *(Hiëraaëtus fasciatus)*	1	1454	875	71	37.91	3.29	2.30	0.92
Golden eagle *(Aquila chrysaëtos)*	1	1232	138	–	13.52	3.71	1.73	2.64
Lanner falcon *(Falco biarmicus)*	2	2111 / 1340	132/traces	traces	40.74 / 19.38	3.67 / 2.46	2.51 / 1.69	1.09 / 1.05
Saker falcon *(Falco chergus)*	1	1440	472	64	1.95	5.41	2.24	2.54
Peregrine falcon *(Falco peregrinus)*	3	1026 ± 297	86 ± 135	traces	5.30 ± 5.37	4.94 ± 1.30	2.68 ± 0.49	1.52 ± 0.68
Common kestrel *(Falco tinnunculus)*	2	1194 / 821	577 / 505	traces	27.45 / 8.97	3.74 / 3.42	1.99 / 1.40	1.57 / 1.32
Galliformes								
Chicken *(Gallus gallus domesticus)*	10*	357 ± 96	–	–	2.86 ± 1.28	2.07 ± 0.28	1.33 ± 0.47	0.37 ± 0.05
	7**	797 ± 214	traces	–	4.10 ± 2.02	4.57 ± 1.97	0.85 ± 0.29	11.42 ± 6.09
Quail *(Coturnix coturnix)*	11	817 ± 127	–	–	1.74 ± 0.69	5.03 ± 0.53	1.26 ± 0.16	7.13 ± 1.43
Turkey *(Meleagris gallopavo)*	12	1099 ± 327	traces	–	2.40 ± 0.98	2.20 ± 0.36	1.30 ± 0.28	0.53 ± 0.22
Gruiformes								
Red-crowned crane *(Grus japonensis)* (ZH)	1	101	–	–	1.04	nd	nd	nd
Strigiformes								
Eagle-owl *(Bubo bubo)* (GK)	4	299 ± 67	traces	–	0.79 ± 0.60	1.86 ± 0.83	1.06 ± 0.28	2.38 ± 0.83
Tawny owl *(Strix aluco)* (F)	1	526	traces	–	20.64	nd	nd	nd

The amounts of retinyl esters in plasma are expressed as the equivalent weight of retinol. Code: ZH = Zoological garden Hellabrunn — München; ZN = zoological garden Nürnberg; GK = Institut für Geflügelkrankheiten, Veterinary Faculty München; F = Falconry. * imature chickens; ** mature female chickens; nd = not determined. The [illegible]

Lipids as well as the fat-soluble vitamins A and E have to be transported in the aqueous environment of blood by protein carriers. For the investigated plasma components two different types of carrier exist. Vitamin E as well as the lipids are transported by the lipoproteins with a species specific distribution among the lipoprotein fractions (Kirkeby, 1966; Galster et al., 1977; Leat et al., 1979). For vitamin A, a specific carrier protein — the retinol-binding protein (RBP) — exists. This carrier protein is of importance for the homeostatic level of retinol, the physiological transport form of vitamin A, in the blood. Because of the homeostasis of retinol in the blood, changes in vitamin A levels are only observed if either stores (liver) are heavily depleted or animals suffer from hypervitaminosis A (Smith and Goodman, 1979). Contrary to this, vitamin E in the blood, carried by lipoproteins, is clearly affected by the intake of vitamin E with the food (Willett et al., 1983) as well as the lipid levels in the blood (Davies et al., 1969). Therefore, vitamin E status can be monitored by measuring the vitamin E plasma level, while vitamin A levels are less useful in the assessment of the vitamin status.

We were the first to show that, contrary to the assumption that lipoprotein-bound retinyl esters are responsible for the signs of vitamin A intoxication (Mallia et al., 1975; Smith and Goodman, 1976), in a whole order of mammals (Carnivora) a high percentage of retinyl esters is bound to plasma lipoproteins and seems to be a physiological way of vitamin A transport (Schweigert, 1988; Schweigert et al., 1990). This study now shows, that in most of the investigated species, only retinol — very probably bound to the specific carrier, the RBP — was present in plasma. The levels observed (< 500 ng/ml) were in the order of magnitude of levels reported by others (Baker et al., 1986; Baker et al., 1988; Ghebremeskel and Williams, 1988). It is interesting to note that in bats which were obviously healthy, only trace amounts of retinol were detected. Further investigations would be needed to determine whether, in the animal kingdom, this indicates another peculiarity in vitamin A metabolism. In birds, retinol values were higher ranging from 500 to 2,000 ng/ml and in addition, similar to carnivores, in most species of the order Ciconiiformes and Falconiformes, retinyl esters (mainly retinyl palmitate/oleate) were detected ranging between 10 to 50 % of total vitamin A (calculated as retinol equivalents) in plasma. Similar to carnivores, birds with a high percentage of retinyl esters in the blood showed no signs of a vitamin A intoxication. The similarity between carnivorous mammals and birds with regard to the occurrence of vitamin A esters in the blood might indicate that the occurrence of vitamin A esters, probably bound to lipoproteins (Ganguly et al., 1952), is an adaptation of vitamin A metabolism to an ample supply with vitamin A in a carnivorous diet. The high percentage of retinyl esters in blood plasma of the Bonelli's eagle, the golden eagle and the common kestrel, however, may be caused by the application of Tricrescovit® (Rhone Merieux) a vitamin preparation. Contrary to previous reports on the vitamin A transport in the blood plasma of chickens, where retinyl esters represented 75 % of total vitamin A (Ganguly et al., 1952) no vitamin A esters could be detected in this study. Even in mature female chickens only traces of esters were present despite a drastic increase in the total plasma lipids due to changes in lipid metabolism with the onset of egg laying which is responsible for the observed high lipid levels (especially triglycerides) in quails too (Schjeide, 1963).

β-Carotene was only found in a few species in the order of Artiodactyla and Perissodactyla. These differences between white- and yellow-fat species are summarized by Goodwin (1984). It can only be speculated that the occurrence of white- and yellow-fat species within the same family — despite comparable feed — might be due to specific intracellular carrier proteins in the enterocytes (Ganguly et al., 1959) limited to only a few species in which the local supplementation of peripheral target tissue may be of importance e. g. in fertility (Schweigert and Zucker, 1988).

Since vitamin E levels in plasma are readily affected by the vitamin E supplementation and lipid levels in blood (Davies et al., 1969; Willett et al., 1983), results for vitamin E were highly variable but comparable to values obtained by others (Caravaggi, 1969; Liu et al., 1983; Baker et al., 1986; Baker et al., 1988; Dierenfeld and Dolensek, 1988;

DIERENFELD et al., 1989). Higher values in primates compared to the other mammals are similar to other primates (AUSMAN and HAYES, 1974; MCGUIRE et al., 1989) and comparable to those found in man (VUILLEUMIER et al., 1983; WILLETT et al., 1983). The much higher levels in birds (1 to 26 µg/ml) compared to mammals correspond to known data (CALLE et al., 1989) may be due to much higher lipid levels in plasma, since both are strongly dependant (DAVIES et al., 1969).

In most of the investigated species, phospholipids represented the majority of lipids followed by cholesterol and triglycerides. Both, the rather high concentration of lipids in birds and the phospholipids as dominating fraction of total lipids, correspond to known data obtained from a variety of species (DANGERFIELD et al., 1976). Lipid values found for the African or Indian elephant correspond well to those obtained from wild animals (BROWN and WHITE, 1979).

Nevertheless, despite the above mentioned variables, certain tendencies within different orders are obvious for fat-soluble vitamins as well as for lipids. For vitamin A, plasma levels in the majority of species ranged from 100 to 500 ng/ml. Lower levels were found in bats, manatees and their closest terrestrial relatives the elephants. In birds and carnivores (SCHWEIGERT et al., 1990), much higher retinol levels can be observed. Especially the occurrence of vitamin A esters in carnivores, as well as in certain orders of birds should not be interpreted as signs of vitamin A oversupplementation. For vitamin E, the known relationship of plasma vitamin E levels with lipid levels in the blood have to be considered when vitamin E status is assessed by measuring the plasma levels of vitamin E. Thus, species with low lipid levels tend to lower vitamin E levels (ungulates, rodents, Marsupalia and Proboscidea) compared to those with high lipid levels such as primates, carnivores, pinnipeds and birds.

Acknowledgements

We would like to thank the following persons and institutions for supplying us with samples: Dr. A. GAUKLER (Zoo Nürnberg), Mr. V. WALTER (Jagdfalkenhof Schloß Rosenburg), Dr. M. VATER (Zoologisches Institut der Ludwig-Maximilians-Universität München), Dr. C. I. FLURER, and Dr. F. GRIMM (Institut für Geflügelkrankheiten, Tierärztliche Fakultät der LMU-München). This work was supported by Hoffmann-La Roche, Basel, Switzerland.

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