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Concentration of carotenoids, retinol and α-tocopherol in plasma of six microchiroptera species

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Abstract

To adequately feed species in captivity it is necessary to know their nutritional habits and their natural availability of specific nutrients. Such essential nutrients are vitamin A, vitamin E and selected carotenoids as vitamin-A-precursors. Because their blood plasma concentration are valid biomarkers of nutritional status of dietary intake, we determined the concentrations of carotenoids, retinol and α-tocopherol by HPLC as well as the transport proteins for retinol, the retinol-binding protein (RBP) and transthyretin (TTR) immunologically in the plasma of six species of microchiroptera from free-ranging animals and compared it in one species (*Carollia perspicillata*) to a group held in captivity. Plasma concentrations of the investigated components were generally much lower compared to most other mammals. Within the bats, differences were observed for all components. As in other species retinol, RBP and TTR were present but no retinyl esters could be detected. Plasma of the insectivorous bat species *Molossus molossus* contained carotenoids. Within the group of carotenoids, β-carotene was dominant and only traces of lutein were present. *Phyllostomus hastatus* revealed the highest α-tocopherol concentration. No differences in the plasma content of the investigated compounds were found between a group of *Carollia perspicillata* kept in captivity for 20 years and free-ranging individuals from a population in Central America. No sex related differences were obvious. In conclusion, nutritional biomarkers in bats were highly variable due to dietary and possible species-specific differences.

Keywords: α-Tocopherol; Bats; Carotenoids; Blood; Microchiroptera; Retinol; Retinol-binding protein; Transthyretin

1. Introduction

Retinol and α-tocopherol play essential roles in the metabolism of mammals. Retinol is of particular importance for vision, fetal development as well as the regulation of proliferation and differentiation of cells during the whole life cycle (Blomhoff et al., 1990). α-Tocopherol has mainly antioxidant properties and protects especially red blood cells, nerve and muscle cells against oxidative damage (Machlin, 1984). Retinol is not produced by plants, and phytophagous animals obtain retinol through the conversion of carotenoids with provitamin A activity (Blomhoff et al., 1990). On the other hand, α-tocopherol, the vitamin E isomer with the highest biological activity, is found both in plant products (especially in vegetable oils) as well as in food of animal origin (Machlin, 1984).

Recent investigations showed that the transport of retinol from the liver to target cells differs among species. In most species retinol is transported mainly by a complex of retinol-binding protein (RBP) and transthyretin (TTR) (Gaetani et al., 2002). The complex formation guarantees that plasma retinol concentrations are maintained in narrow limits. Only traces of retinyl esters are usually measurable (Schweigert et al., 1990; Schweigert et al., 1991). In contrast, blood plasma of canines and mustelids contains high concentrations of retinyl esters (Schweigert et al., 1990). These retinyl esters are associated with all fractions of lipoproteins. α-Tocopherol, on the other

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hand, is usually bound primarily to the β-lipoprotein fraction in blood cells (Machlin, 1984).

In contrast to retinol, blood plasma concentrations of carotenoids and α-tocopherol are useful biomarkers to evaluate the nutrient supplementation in mammals because their concentrations in blood plasma strongly depend on nutrient supply (Sauberlich et al., 1973). The few data available on fat-soluble vitamins in plasma of microchiroptera originate mostly from animals held in captivity (Dierenfeld and Seyjagat, 2000; Heard et al., 1996; Schweigert et al., 1991). The observed concentrations of retinol and α-tocopherol were relatively lower than in other animal taxa. As bats use various nutrition strategies such as insectivory, frugivory, nectarivory, carnivory, sanguinivory or a combination of them (reviewed in Neuweiler, 1990), species-specific variations in retinol and α-tocopherol metabolism in response to different feeding habits may be expected.

The aim of the study was to determine the content of carotenoids, retinol, retinyl esters and α-tocopherol in the plasma of frugivorous and insectivorous, healthy, free-ranging bats as well as to compare these parameters between free-ranging and captive individuals in one species namely *Carollia perspicillata*. So far, nothing is known about the retinol transport in bat species. Therefore, the occurrence of the transport proteins for retinol (RBP and TTR) in blood plasma was also investigated.

2. Materials and methods

2.1. Animals

Six bat species were included in our study (Table 1). Among the family *Phyllostomidae*, three mostly frugivorous species, *C. perspicillata*, *C. castanea* and *C. sowelli* (former name *C. brevicauda*; Hoffmann and Baker, 2003) and two mostly omnivorous species *Phyllostomus hastatus*, *P. discolor* were investigated. For comparative reasons we included the strictly insectivorous species *Molossus molossus* (*Molossidae*) in the study as well.

The field study was carried out at La Selva Biological Station (LS, 10°25′52″ N, 84°00′12″ W) in Costa Rica. LS is located in the Caribbean lowland at the base of the Cordillera Central at an elevation of 30 to 100 m above sea level. The nature reserve covers a total of 1600 ha of forest, including ca. 600 ha of primary forest connected to the Braulio Carillo National Park (46,000 ha). Habitat types have been classified by Hartshorn and Hammel (1994). Monthly rainfall averages 333 mm with a pronounced dry period between February and April. The bats were caught with a mist net (70 dernier/2 ply, 16 mm mesh, 5 shelves, R. Vohwinkel GmbH, Velbert, Germany) during 14 occasions in March 2006 (dry season) from 6 to 9 pm. Species, sex and reproductive condition were recorded. Age was determined on the basis of closure of the phalangeal epiphyses.

Table 1
Bat species, origin, number and sex of studied individuals

Species	Origin	*n*	Sex	
			Male	Female
Carollia perspicillata	Captive	10	4	6
Carollia perspicillata	Free-ranging	5	3	2
Carollia castanea	Free-ranging	17	11	6
Carollia sowelli	Free-ranging	2	1	1
Phyllostomus hastatus	Free-ranging	4	0	4
Phyllostomus discolor	Free-ranging	1	0	1
Molossus molossus	Free-ranging	6	4	2

The ten captive animals belong to a group of 40 *C. perspicillata* (20 male/20 female) originating from individuals captured in the wild about 20 years ago. They were sampled during a health check. The group is held in a flight room (6×3×3 m) at 28 °C, 80% relative humidity, and at a light cycle of 12:12 h. During the day, light was provided by a 15 W red light bulb (Philips partylight A 60). During the daily feeding and cleaning routine a 60 W incandescent lamp was used. The animals were fed once a day a diet (U. Schmidt; K.-H. Esser pers. comm.) consisting mainly of bananas, oat flakes, one spoon honey and one drop multivitamin solution (Multibionta®, Merck: 1 mL solution containing 2.75 mg retinyl palmitate (5000 IU vitamin A); 4 mg α-tocopherol acetate) resulting in a total mass of 1.0–1.5 kg for the whole group. Twice a week the bats received bananas mixed with milk pudding (Milupa Früchtebrei®, Friedrichsdorf, Germany) with a droplet of Kanavit® (Medphano; 1 mL containing 20 mg phytomenadione) plus one third of teaspoon of minerals (Provit Aufbaukalk®, containing additionally 25000 IU/kg vitamin A and 1000 mg/kg vitamin E) resulting in a mass of 1.0 kg for the whole group. Two animals of the studied captive *C. perspicillata* were juveniles, the others were adults. The captive animals were sampled in February. All animals studied in the field were adults and were sampled in March during the dry season when reproduction takes place. None of the female bats were pregnant except for two *M. molossus*. All animals except one male *M. molossus* were in good body condition.

2.2. Blood sampling

Blood sampling of the captive bats was performed on one day between 11 and 12 am just before feeding. The sampling of the captured bats was conducted 30 min after capture at the beginning of the active phase between 6 to 9 pm on several days.

Approximately 60 μL blood were taken from all animals by puncturing the propatagial vein and sampling the blood drops in a heparinized microcapillary tube (75 mm, Brand GmbH, Wertheim, Germany) (Voigt and Cruz-Neto, in press). The blood samples were transferred into small Eppendorf tubes and centrifuged for 10 min (Galaxy mini centrifuge, Merck Eurolab, Leuven, Belgium). Plasma was separated and stored at −80 °C until analysis. All free-ranging bats were released after blood sampling.

2.3. HPLC-analysis

The samples were analysed within three weeks after acquisition. The analysis of carotenoids, retinol and α-tocopherol was conducted using a modified gradient reverse-phase, high-performance liquid chromatography system (Waters HPLC System, Waters, Eschborn Germany) as described elsewhere (Schweigert et al., 2000). Briefly, carotenoids, retinol and α-tocopherol were extracted from plasma and separated on a