The Journal of Experimental Biology 206, 1419-1427
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doi:10.1242/jeb.00274



# Low turnover rates of carbon isotopes in tissues of two nectar-feeding bat species

Christian C. Voigt[1,*], Felix Matt[2], Robert Michener[3] and Thomas H. Kunz[3,4]

[1]*Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany,* [2]*Zoologisches Institut II, Universität Erlangen-Nürnberg, Staudtstr. 5, 91058 Erlangen, Germany,* [3]*Stable Isotope Laboratory, Department of Biology, Boston University, Boston, MA 02215, USA* and [4]*Center for Ecology and Conservation Biology, Department of Biology, Boston University, Boston, MA 02215, USA*

*Author for correspondence (e-mail: voigt@izw-berlin.de)

*Accepted 29 January 2003*

## Summary

**Stable isotopes of carbon are commonly used to characterize dietary preferences in animals. Because turnover rates of carbon isotopes are related to metabolic rate, we wanted to determine the rates at which carbon isotopes are exchanged in tissues of two species of nectar-feeding bats (*Leptonycteris curasoae* and *Glossophaga soricina*), both of which have relatively high mass-specific metabolic rates. To test the hypothesis that isotope turnover is higher in nectar-feeding bats, because of their high mass-specific metabolic rates, than in other eutherian mammals, we conducted diet-switching experiments and chose three target tissues (hair, wing membrane and blood) to evaluate the isotopic turnover rates. We made the following predictions: (1) isotopic composition should change towards higher $\delta^{13}$C-values due to the turnover of carbon isotopes of $C_3$ origin with those of $C_4$/CAM origin; (2) the turnover rates of carbon isotopes would differ between the three types of tissues in the following order of decreasing turnover rates: blood>wing membrane>hair; and (3) turnover rates of nectar-feeding bats should exceed those reported for other small mammals because of the high mass-specific metabolic rate of nectar-feeding bats. Compared to the initial diet, target tissues were enriched in heavy carbon isotopes by 2.8‰ in *L. curasoae* and by 2.6‰ in *G. soricina*. After changing the diet from $C_3$ to $C_4$/CAM origin we found an increase in abundance of $^{13}$C in blood and wing membrane in all experimental subjects. The estimated half life of carbon isotope turnover ranged from 100 to 134 days and did not differ significantly between blood and wing membrane, nor did it differ between the two species. The low turnover rate in wing membrane may reflect its specific composition and the relatively low temperature of this tissue, and long-lived erythrocytes in bat blood may be responsible for the low turnover rate of carbon isotopes in blood. The turnover rate of stable carbon isotopes in hair was low in *L. curasoae* and undetectable in *G. soricina*, which may be explained by the seasonal growth of the hair in these two species. Because both species are small (10 and 25 g, respectively) and nectar-feeding bats have higher mass-specific metabolic rates than bats in temperate regions or similar sized terrestrial mammals, our findings of low turnover rates were unexpected.**

Key words: metabolism, carbon isotope, bat, *Leptonycteris curasoae*, *Glossophaga soricina*, fractionation.

## Introduction

Stable isotopes of carbon occur at varying ratios as a consequence of the specific enzymatic route of $CO_2$-fixation ($C_3$ or $C_4$/CAM) in plants and in animals according to their diet (DeNiro and Epstein, 1978, 1981). These observations are the basis for studies on dietary preferences (e.g. Ben-David et al., 1997; Hobson et al., 2000) and migratory movements of animals (e.g. Fleming et al., 1993; Marra et al., 1998; Hobson, 1999). Based on carbon and hydrogen isotope data, Rubenstein et al. (2002) showed, for example, that black-throated blue warblers (*Dendroica caerulescens*) wintering on western Caribbean islands originate from the northern end of the species' range, whereas birds wintering on eastern Caribbean islands are primarily from the southern breeding range. In a similar study, Kelly et al. (2002) used hydrogen isotope data to demonstrate that southern populations of Wilson's warbler (*Wilsonia pusilla*) migrate to the northern range of the wintering habitat, whereas northern populations migrate in a leapfrog pattern to southern wintering areas. It has long been proposed that the analysis of stable isotopes of several tissues from the same animal can serve as a window to different dietary periods, because the turnover rates of isotopes may vary between tissues (Hobson and Sealy, 1991). In captive rodents, for example, turnover rates of carbon isotopes were high in fat and liver, intermediate in muscles and low in brain and hair (Tieszen et al., 1983). Stable isotope composition in fat and liver can give short-term dietary information, that of muscles medium-term dietary information, and brain and hair long-term dietary information. This comparison can be

valuable in the study of migration because it helps to distinguish between individuals that have newly arrived at the wintering habitat from those who arrived earlier (Hobson, 1999). In addition, it may help to track changes in feeding habits or metabolic states (e.g. catabolizing *versus* fat-depositing animals) in dietary studies.

Tieszen et al. (1983) showed that the isotope turnover rates in tissues are related to the tissue-specific turnover rates. On a whole-animal basis, it could be argued that animals with a high mass-specific energy turnover rate should have a high isotope turnover rate. Nectar-feeding bats (Glossophaginae, Phyllostomidae) have mass-specific metabolic rates that exceed those of most other eutherians by a factor of two (von Helversen and Winter, in press). Thus, we were interested to determine at what rate stable carbon isotopes are exchanged in tissues of nectar-feeding bats. We postulated that the isotope turnover of nectar-feeding bats should be higher than in other eutherian mammals.

Stable isotopes have been used in the study of bats and especially in nectar-feeding bats (Herrera et al., 1993, 1998, 2001; Fleming et al., 1993; Fleming, 1995). Fleming et al. (1993) showed that migratory *Leptonycteris curasoae*, within their northern distribution range, feed extensively or, in some cases even exclusively, on the nectar of CAM-plants (Cactaceae and Agavaceae) with a carbon isotope ratio of approximately –10‰. By contrast, in the southern parts of their distributional range in Mexico, which corresponds to the wintering habitat of northern populations, *Leptonycteris* feeds primarily on nectar of $C_3$ plants that secrete sugar with a carbon isotope ratio of –25‰. By analyzing the carbon isotope abundance in tissues of *L. curasoae* from different geographical regions, Fleming et al. (1993) tracked the seasonal patterns of dietary preferences and migratory movements of this species, and concluded that *L. curasoae* depends on a spatio–temporal nectar corridor of cacti and agave flowers during migration.

We performed a diet-switching experiment with two glossophagine bat species (*Leptonycteris curasoae* and *Glossophaga soricina*), during which we changed the carbon isotope ratio of the diet by almost 14‰. Diet-switching experiments are facilitated in nectar-feeding animals because the isotope ratio of the diet can be changed without changing the overall composition of the diet, as both diets are based on sugar water. We collected three types of tissue (hair, wing membrane and blood) that caused minimal harm to the animals. We selected blood as our standard target tissue, assuming that it would show the highest turnover rate. We chose small pieces of wing membrane because we expected it to exhibit an intermediate turnover rate relative to blood and hair. Biopsies of wing membrane are routinely taken for DNA extraction during genetic studies (Worthington Willmer and Barratt, 1996) and, based on our experience, punctured wing membranes regenerate within 3–4 weeks. We chose hair because of its expected low turnover rate (Tieszen et al., 1983). As a metabolically inert tissue at maturity, isotope ratios in hair should reflect the isotope composition of food consumed only during the time of hair growth.

## Materials and methods

We randomly selected ten *Leptonycteris curasoae* Miller (7 males and 3 females) and ten *Glossophaga soricina* Pallas (3 males and 7 females; Phyllostomidae, Glossophaginae) from a captive breeding colony maintained in greenhouse facilities at the University of Erlangen-Nürnberg, Germany. Female bats were neither pregnant nor lactating. All bats were banded with coloured and numbered plastic bands on the forearm (size XCL for *G. soricina* and XL for *L. curasoae*; A. C. Hughes, Hampton Hill, UK) for individual identification. The animals were introduced into two indoor flight enclosures (4 m×3 m×2.5 m), one for each species, and the two groups were maintained over a period of 70 days with food provided *ad libitum* at three artificial feeders. All bats were returned to the breeding colony after the experiment. The room temperature was set to approximately 23°C, the ambient humidity to 70% and the light:dark regime to 12 h:12 h. The two groups were habituated to the flight enclosure over a 1-week period and fed with the same diet as in the breeding colony. The food provided during the initial phase of the experiment was identical in isotopic composition with the diet the bats received in the greenhouse facility where they were held prior to and after the experiment. This food originated from plants using the $C_3$ photosynthetic pathway (Table 1). The stock solutions (Tables 1, 2) were dissolved in water to a final concentration of 18% sugar water (mass/mass, Atego refractometer; accuracy 0.2%) and the diluted sugary waters were provided from separate feeders.

After 7 days on the initial diet, we switched the diet of plants to one with a carbon isotope composition of the $C_4$ and CAM photosynthetic pathways (Table 2), referred to as day 1 of the experiment. As before, the stock solutions were diluted to a sugar concentration of 18% (mass/mass). To complement the sugary solution of the bats, we added several mg of vitamins and mineral supplements to the diet each day.

Before and after each night, the sugar water was weighed to

Table 1. *Isotopic composition (‰ $\delta^{13}C$) of the diet before the experiment*

| Food source | $\delta^{13}C$ (‰) |
|---|---|
| Alete | –22.7[a] |
| Honey | –26.0[a] |
| Nektar Plus | –25.6±0.3 |
| Pollen | –26.7±0.5 |
| Banana | –23.7[a] |

[a]Single measurement.

Values are means ± S.D.

Alete, honey and Nektar Plus were the three main food sources during the initial week prior to the switch in diet. The food was supplemented by small amounts of pollen and banana.

Table 2. *Isotopic composition (‰ $\delta^{13}C$) of the diet during the experiment*

| Food source | $\delta^{13}C$ (‰) |
|---|---|
| Cane sugar | −10.4±0.1 |
| Corn syrup | −9.7±0.2 |
| Agave syrup | −11.3±0.3 |
| Opuntia fruits | −11.3±0.2 |

Values are means ± S.D.

Cane sugar, corn syrup and agave syrup were the three main food sources starting from day 1 of the experiment. The food was supplemented by small amounts of opuntia fruits.

Table 3. *Comparison of isotopic composition between washed and non-washed hair samples from* Leptonycteris curasoae

| | Isotope composition (‰) | | | |
|---|---|---|---|---|
| Isotope | Non-washed | Washed | *t*-statistics | Significance level |
| $\delta^{13}C$ | −21.6±0.6 | −20.2±0.8 | $t_7$=15.1 | *P*<0.01 |
| $\delta^{15}N$ | 8.0±1.5 | 8.0±1.5 | $t_7$=1.0 | *P*=0.38 |

Values are means ± S.D.

an accuracy of 1 g and we refer to ingested food as the difference between the two measurements. The concentration of $^{13}C$ in the ingested food was estimated by calculating the proportion of each of the three main food sources that were ingested each day and by multiplying this value with the corresponding concentration of $^{13}C$ in the food items (Tables 1 and 2).

At the end of the first week and during each subsequent sampling event, bats were weighed to the nearest 0.01 g on an electronic balance (Mettler PM-100, Columbus, OH, USA) and three types of samples were taken from each bat. We took two tissue samples from the wing membrane using a 3 mm diameter biopsy punch. Next, we drew approximately 30 µl of blood from the propatagial vein using a small sterile needle. Finally, we removed hair from an area of approximately 0.25 $cm^2$ from the back of each bat. The hair was cut with scissors at the base close to the skin and from varying regions of the back but from the same spot at a given day. All samples were placed into Eppendorf tubes, labelled and transferred immediately into a drying oven, where they were dried to constant mass at 60°C. Subsequently, samples were stored in a freezer below 0°C. After changing the diets, we took blood, wing membrane and hair samples at the end of the second, fourth, sixth and eighth weeks.

To remove external contaminants from skin and hair samples, we washed the samples with a chloroform/methanol solvent (1:1). To test for any differences between washed and unwashed samples, we collected samples of hairs from eight *Leptonycteris* and divided each sample into two parts. The first part was washed in the solvent and the second part remained untreated. We then measured the carbon ($\delta^{13}C$, ‰) and nitrogen isotopes ($\delta^{15}N$, ‰) in the hair samples following the procedure described below and performed pair-wise comparisons between washed and unwashed samples. We found a significant carbon enrichment of 1‰ in treated *versus* untreated samples but no difference in $\delta^{15}N$ (Table 3).

*Sample analysis and conversion to the δ notation*

Samples were combusted and the resultant gases ($N_2$ and $CO_2$) were sequentially measured in a CE 1110 elemental analyzer connected *via* a continuous flow system to a Thermo Finnigan Delta Plus isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany). The sample isotope ratios were compared with international gas standards (USGS-24 and IAEA-N1). Precision was better than ±0.1‰ for both nitrogen and carbon. Isotope ratios are expressed in the δ notation in parts per thousand (‰) using the following equation for carbon isotopes:

$$\delta^{13}C = [(^{13}C/^{12}C)_{sample}] / [(^{13}C/^{12}C)_{standard} - 1] \times 10^3 , \quad (1)$$

and for nitrogen isotopes:

$$\delta^{15}N = [(^{15}N/^{14}N)_{sample}] / [(^{15}N/^{14}N)_{standard} - 1] \times 10^3 . \quad (2)$$

We used the carbon isotope ratio of Vienna Pee Dee Belemnite limestone and the nitrogen isotope ratio of air as standards.

*Statistical analysis and curve estimation*

To test for differences in mean isotope enrichment between (1) the tissues and (2) the diets, we performed one-way analysis of variance (ANOVA). We ran *post-hoc* Tukey HSD tests for pair-wise comparisons to evaluate differences in mean values.

We calculated mean isotopic values for all sample periods. In theory, changes in isotopic composition should follow an exponential curve (e.g. Tieszen et al., 1983). Hence, equations of the type $y=a+be^{ct}$ were fitted to the $\delta^{13}C$ data from each tissue and each bat species. In this equation, $a$ represents the asymptotic $\delta^{13}C$ value for the tissue equilibrated on a $C_4$/CAM-diet, $b$ equals the overall change in isotope ratio, $c$ is the turnover rate of carbon isotopes in the tissues, and $y$ the mean carbon isotope ratio in the tissue at the time $t$. For the reasons of simplicity, we refer to $c$ as the regression coefficient in the exponential model.

We assumed that the different tissues equilibrate to an isotope ratio close to the value measured for the $C_4$/CAM-diet plus the average fractionation value found for that specific tissue. Thus, $a$ equalled the average isotope ratio of carbon isotopes in the $C_4$/CAM-diet plus the difference between $\delta^{13}C$ of the $C_3$ diet and the tissue caused by fractionation. Additionally, we assumed that $b$ equalled the overall change in isotopic composition in the two diets, which was 13.7‰ in *Leptonycteris curasoae* and 13.6‰ in *Glossophaga soricina*. Estimation of $c$ was performed on an iterative basis starting with a value of 0.05. The iteration was stopped after changes in the sum of squares were smaller than $1.0\times10^{-8}$. To estimate the half time of the carbon isotope exchange in the different tissues, we calculated $t_{50}$ using the following equation:

$t_{50}$=log$_e$(0.5)/$c$, where $t_{50}$ is the time in days in which half of the carbon isotopes were exchanged in the corresponding tissue, and 0.5 represents the exchange of 50% isotopes. For reasons of simplicity we will describe the half life of carbon isotopes (=$t_{50}$) in hair, although we are aware that the isotope composition of hair reflects only the period of growth, as hair is an isotopically inert tissue.

### *Possible effect of body mass changes on regression coefficients*

In a preliminary experiment with five *Leptonycteris curasoae*, we observed that within 22 days following the diet switch bats had lost some body mass. To evaluate a possible bias in our experiment due to loss of body mass, we calculated exponential exchange curves on an individual basis. We then tested whether the rate of increase $c$ of the exponential functions was related to the change in body mass. We predicted that the regression coefficient $c$ decreased with increasing loss of body mass if carbon isotopes of catabolized fat or protein of $C_3$ origin mixed with the ingested carbon isotopes of $C_4$/CAM origin. We expected such effects to be most apparent in tissues with a relatively high turnover and thus performed the statistical analysis only for the data set of wing membrane and blood. The level of significance was Bonferroni-corrected to 2.5%, because two data sets were tested for each individual.

Values are expressed as means ± 1 S.D. In general, two-tailed tests were performed. We used SPSS (version 9.0) for all statistical analysis and regression models.

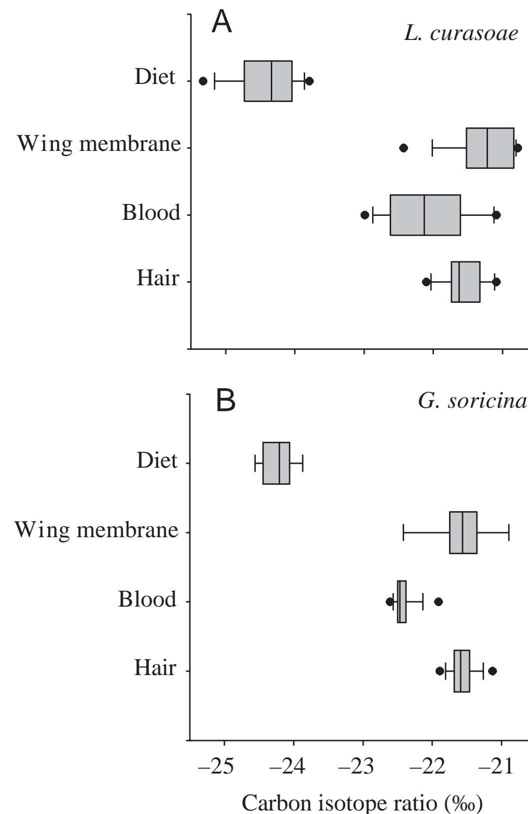


Fig. 1. Carbon isotope ratios ($\delta^{13}$C; ‰) in wing membrane, blood and hair of *Leptonycteris curosoae* (A) and *Glossophaga soricina* (B) compared to the initial diet originating from plants with a $C_3$ photosynthetic pathway. Data are presented as box plots with the border of the box representing the 25% and 75% percentiles, the T-mark shows the 10% and 90% percentiles and the outermost points the 5% and 95% percentiles. The thin line within the box indicates the median.

## Results

### *Fractionation of isotopes*

At the end of the initial phase of the experiment (i.e. before day 1), mean values of isotopic composition differed significantly between the first diet and the three target tissues (Fig. 1; ANOVA: *Leptonycteris curasoae*: $F_{3,33}$=66, $P$<0.001; *Glossophaga soricina*: $F_{3,33}$=98, $P$<0.001). The difference between carbon isotope ratios of the initial diet and in the animal tissues averaged 2.8±0.4‰ for *L. curasoae* and 2.6±0.5‰ for *G. soricina*. In *L. curasoae*, blood was more enriched in heavy carbon isotopes than wing membrane (Fig. 1: Tukey's test, $P$=0.007). In *G. soricina*, blood contained more $^{13}$C than wing membrane and hair (Fig. 1: Tukey's test, $P$<0.001).

### *Nutritional intake and body mass changes*

On average, individuals of *L. curasoae* ingested more sugar water per day (20.3±2.9 ml day$^{-1}$) than did individuals of *G. soricina* (19.2±1.8 ml day$^{-1}$) (Student $t$-test, $t_{110}$=2.6, $P$=0.014). Daily rate of food intake did not vary between diets 1 and 2 (*L. curasoae*: Student $t$-test: $t_{61}$=1.95, $P$=0.06, *G. soricina*: Student $t$-test, $t_{61}$=0.23, $P$=0.82). In addition, the mean nutritional intake rate remained constant throughout the experiment (ANCOVA: *L. curasoae*: $F_{4,58}$=1.4, $P$=0.25; *G. soricina*: $F_{4,58}$=0.97, $P$=0.43). In both species, mean body mass decreased significantly during the course of the experiment (ANOVA for repeated measures: *L. curasoae*, interval: $F_{4,39}$=37, $P$<0.001; *G. soricina*, interval: $F_{4,39}$=63, $P$<0.001). In addition, mean body mass was significantly different between individuals (*L. curasoae*, $F_{9,39}$=26, $P$<0.001; *G. soricina*, individual, $F_{9,39}$=43, $P$=0<0.001). On day 1 of the experiment, the mean body mass of *L. curasoae* was 23.6±2.1 g and *G. soricina*, 10.2±0.7 g. At the end of the experiment, mean body mass of *L. curasoae* had decreased to 21.7±2.3 g and of *G. soricina* to 9.4±1.0 g. Thus, both species lost on average 8% of their initial body mass during the course of the experiment. Body mass changes of individuals of both species are plotted in Fig. 2A,B.

### *Changes of isotope abundance during the experiment*

After switching the diet to plant products of $C_4$/CAM-origin, the enrichment of heavy carbon isotopes increased in all three types of tissues sampled in both species (Fig. 3A,C,E for *Leptonycteris curasoae* and Fig. 3B,D,F for *Glossophaga soricina*). At the end of the experiment, at day 60, none of the tissues had equilibrated to the expected point of carbon isotopic enrichment (first numerical value in the exponential regression

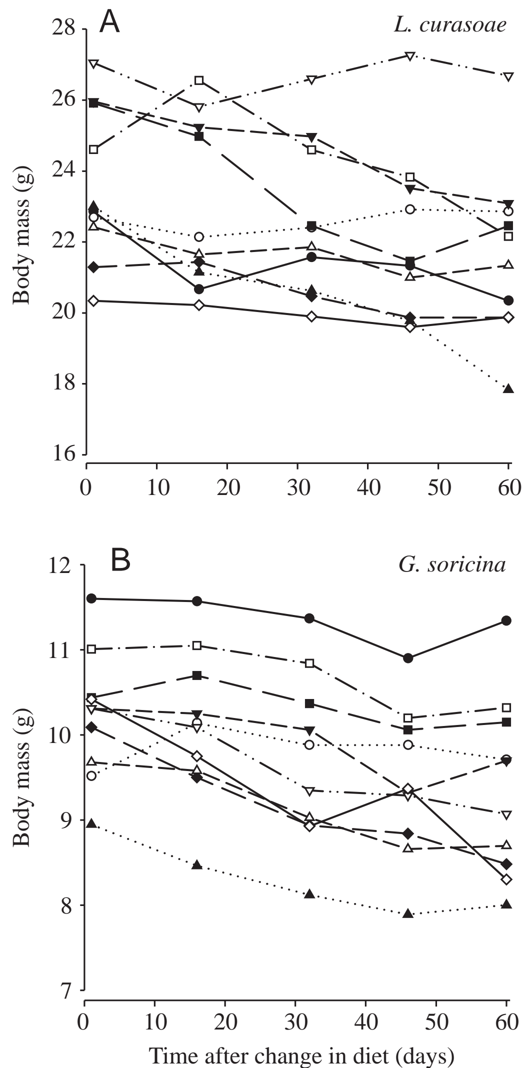


Fig. 2. Mean body mass (g) of individual *Leptonycteris curasoae* and *Glossophaga soricina* during the course of the experiment (days). Individuals are presented as different symbols; data points of the same individuals are connected by lines. Day 1 represents the onset of the experiment (i.e. the switch in diet from the $C_3$ to $C_4$/CAM origin).

equations plotted in the graphs). Based on the calculated exponential equations, we estimated the half life of the carbon isotopes in tissues (see $t_{50}$ values in Figs 3 and 4). In both species, wing membrane and blood had very similar turnover rates of approximately 116 days (range: 102–134 days, Table 4). Based on a paired *t*-test, mean regression coefficients of wing membrane and blood were not significantly different within species (*L. curasoae*: $t_9$=1.0, *P*=0.32, *G. soricina*: $t_9$=1.3, *P*=0.22). In addition, neither mean regression coefficients of blood nor those of wing membrane were different between species (Student *t*-test; blood: $t_{18}$=0.1, *P*=0.91; wing membrane: $t_{18}$=1.4, *P*=0.17). Compared to blood and wing membrane, estimated $t_{50}$ values for hair were higher by a factor of five, averaging 537 days in *L. curasoae* (Table 4). We could not detect a significant change in $\delta^{13}$C in hairs of *G. soricina*.

Table 4. *Estimated time intervals (days) during which half of the carbon isotopes were exchanged in the three different tissues under study*

| | Time interval (days) | | | |
|---|---|---|---|---|
| | *L. curasoae* | | *G. soricina* | |
| Tissue | Mean | 95% CI | Mean | 95% CI |
| Wing membrane | 134 | 115–160 | 102 | 80–143 |
| Blood | 120 | 105–140 | 113d[a] | 107–121 |
| Hair | 537 | 396–836 | n.s. | |

95% CI, 95% confidence intervals; n.s., not significant.

[a]When compensating for body mass loss in *G. soricina*, blood $t_{50}$=126 days (see Fig. 4).

Table 5. *Correlation coefficients for the relationship between individual regression coefficients and body mass losses in* Leptonycteris curasoae *and* Glossophaga soricina

| Species | Sample type | Correlation coefficient (*r*) | *P*-value |
|---|---|---|---|
| *L. curasoae* | Blood | 0.66 | 0.036 |
| *L. curasoae* | Wing membrane | 0.45 | 0.197 |
| *G. soricina* | Blood | –0.87 | 0.001 |
| *G. soricina* | Wing membrane | –0.10 | 0.790 |

The level of significance was Bonferroni-corrected to a value of 2.5%.

### *Effect of body mass loss on isotope turnover rates*

On an individual basis, we tested whether body mass loss was related to the regression coefficient of the exchange curve of carbon isotopes in *Leptonycteris curasoae* (Fig. 4A) and *Glossophaga soricina* (Fig. 4B). In blood samples of *G. soricina*, the regression coefficients increased significantly with increasing loss of body mass of the corresponding individual (Table 5). Thus, contrary to our expectation, turnover rates were faster in those animals that lost body mass than those with a constant body mass, at least in blood of *G. soricina.* When controlling for the effect of loss of body mass in *G. soricina*, $t_{50}$ of blood was 126 days (see legend of Fig. 4).

## Discussion

During biochemical processes, enzymes discriminate against heavy isotopes of carbon and nitrogen (DeNiro and Epstein, 1978, 1981). In addition to the processes of kinetic fractionation, this leads to an average enrichment of heavy nitrogen of 3.4‰ between trophic levels (Eggers and Jones, 2000). The discrimination function is less intense for carbon isotopes than for nitrogen isotopes and, thus, the enrichment of $^{13}$C from diet to tissue is smaller: on average only 1‰ or 2‰ (DeNiro and Epstein, 1981). Hobson and Clark (1992b) reported values from 0.2‰ to 2.7‰ in tissues of Japanese quails (*Coturnix japonica*). In our study, the fractionation

effect against $^{13}C$ at 2.8‰ and 2.7‰ was consistent within both species and similar to the findings of Hobson and Clark.

We found a low rate of growth in hair of *Leptonycteris curasoae* and no significant change in the isotope composition of *Glossophaga soricina* hair. Because hair is a metabolically inert tissue, only the basal, growing part should reflect the isotope ratio of the current diet. Thus far, all bats studied with respect to hair growth and molt showed a seasonal pattern (Constantine, 1957, 1958; Kunz, 1974; Mazak, 1963, 1965). Thus, if both study species were in a phase of reduced hair growth, only negligible amounts of carbon with a $C_4$ isotope signature would be included. Since the patterns of hair growth are unknown for glossophagine bats, we cannot evaluate the effects of seasonal hair growth in our study species.

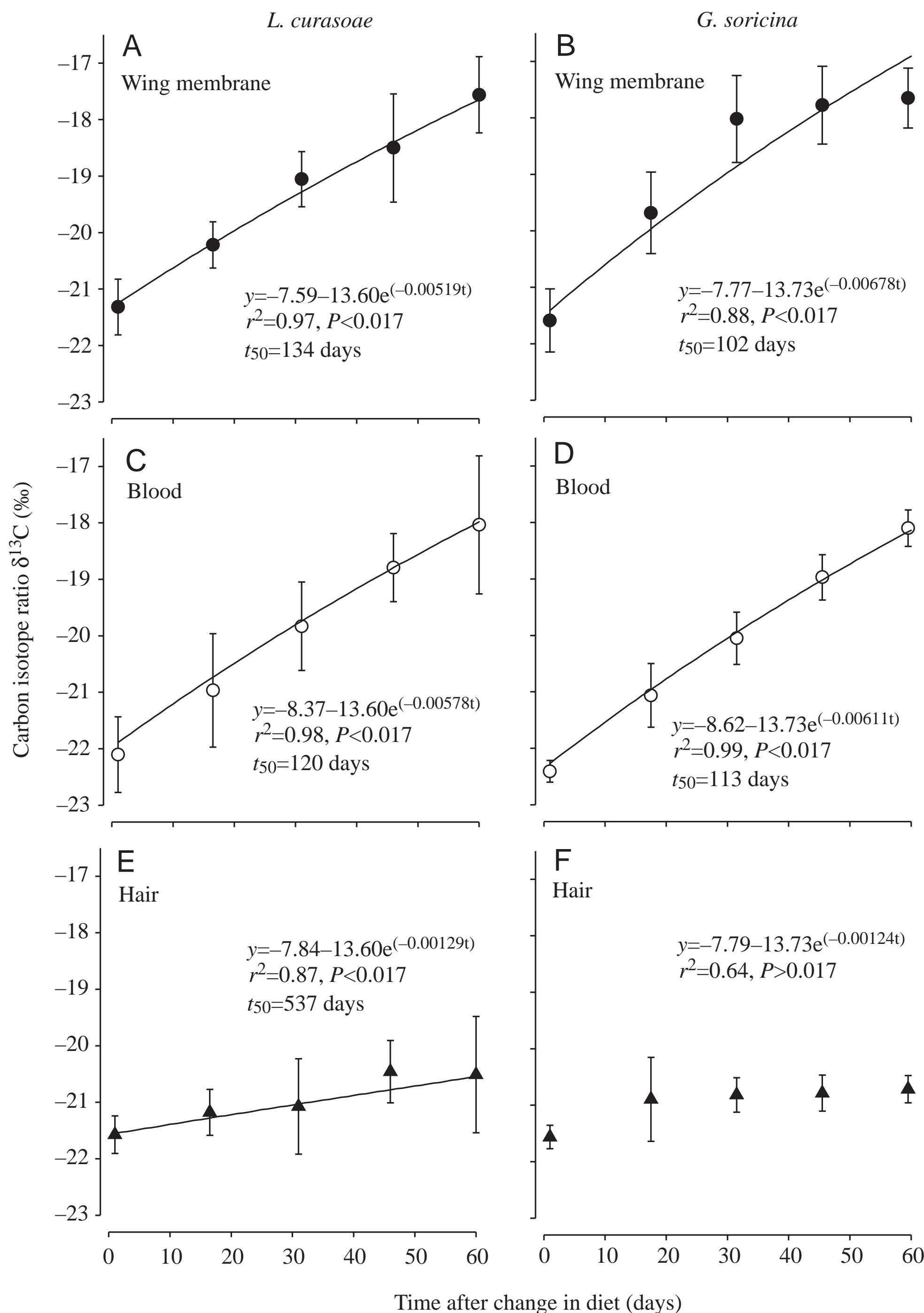


Fig. 3. Changes in carbon isotope ratio ($\delta^{13}C$; ‰) in three tissues, wing membranes (A,B; solid circles), blood (C,D; open circles) and hair (E,F; triangles) of *Leptonycteris curasoa* (A,C,E) and *Glossophaga soricina* (B,D,F) after the diet was changed at day 1 from the $C_3$ source to products originating from plants representative of the $C_4$/Cam photosynthetic pathway. Values are means ± 1 S.D. Exponential regression functions were fitted to the data sets as indicated.

In the wing membrane of bats, the estimated half life of carbon isotopes was 100 and 130 days. In contrast to this finding, biopsies of wing membranes heal at a remarkably rapid rate (see also Worthington Wilmer and Barratt, 1996). In nectar-feeding bats, punctured wing membranes caused by a biopsy punch heal and completely close within 3–4 weeks. However, the regeneration time of damaged bat wings is probably not representative of the regeneration time of the whole tissue. Quite likely, special biochemical processes are triggered after a wing membrane is damaged and, as a consequence, regeneration time of wounds in the membrane is higher than the overall turnover time in that tissue. Wing membranes comprise an extremely reduced dermis that is sandwiched between the dorsal and ventral layer of the epidermis (Quay, 1970). To increase elasticity and stiffness, bat wings consist of a two-dimensional network of large, macroscopic collagen–elastin fibre bundles (Holbrook and Odland, 1978). Palaeontologists use bone collagen as an indicator of yearly or lifetime diets of organisms because collagen is known to regenerate at a low rate (e.g. $t_{50}$=173 days in quails: Hobson and Clark, 1992a). High concentrations of collagen and elastin in wing membranes and their possible low rate of regeneration could explain our findings of a low carbon isotope turnover rate in the wing

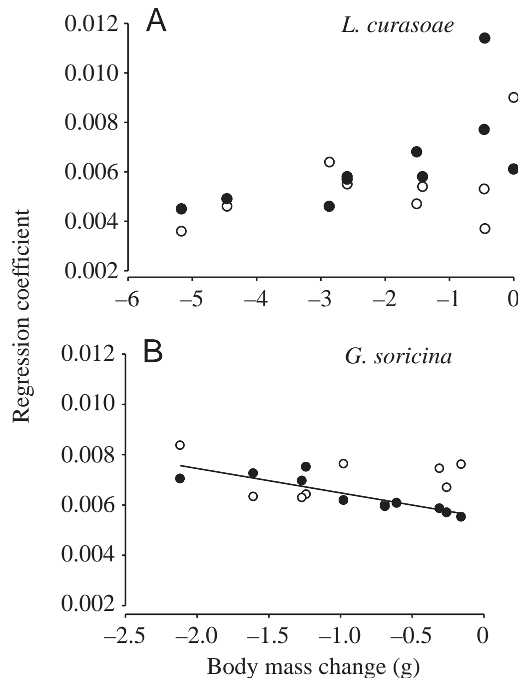


Fig. 4. Relationship between regression coefficients and loss of body mass in individual *Leptonycteris curasoa* (A) and *Glossophaga soricina* (B). Filled circles, blood; open circles, wing membrane. The regression coefficient was significantly correlated with loss of body mass in *G. soricina* from which blood samples were taken (B, solid line). The linear regression equation was $y$=–0.001$x$+0.0055, based on a least-squares analysis.

membrane. Bat wings are also cooler than the core body temperature of bats. Lancaster et al. (1997) showed that the temperature of the wing membrane is approximately 1–2°C above the ambient temperature. Thus, in the present study, biochemical processes in the wing membrane probably took place at a temperature of 24–25°C. The specific molecular composition and the relatively low temperature could explain the low turnover rates of carbon isotopes in bat wings.

Blood was estimated to have a $t_{50}$ value of 120 and 126 days. Because this is the first measurement of blood turnover in bats, we compared our data with those from mammals other than bats. In humans, erythrocytes have a $t_{50}$ value of 60 days (Schmidt and Thews, 1995). In avian blood, the carbon isotope half life was 11 days in Japanese quails (*Coturnix japonica*; Hobson and Clark, 1992a) and approximately 30 days in young, growing American crows (*Corvus brachyrhynchos*; Hobson and Clark, 1993). Our results indicate that the estimated isotope half-life of approximately 120 days in bat blood is higher than corresponding values from other animals, including humans, and is also in contrast to our prediction.

We discuss this unexpected result in the context of three different scenarios. (1) The rate of isotope turnover was slowed down because animals were torpid or lethargic. (2) The bats accumulated a nutritional deficit, which contributed to a loss of body mass. In the latter situation, the mobilization of fat and/or body proteins would affect the estimate of stable isotope turnover rates, so we evaluated the possibility that carbon from sugar and carbon from body tissues are used for different purposes: energy *versus* tissue synthesis. Finally, we consider the possibility that the measured turnover rates of carbon isotopes are representative for the actual tissue turnover despite the presence of body mass loss.

Many bat species are known for their propensity to enter torpor under low ambient temperatures or unfavourable food regimes. Torpor or lethargic conditions have been mostly studied in bats of temperate zones, namely vespertilionid species (see review by Speakman and Thomas, in press). Most tropical bats do not enter torpor on a regular basis, although studies are scarce on this phenomenon in tropical species. Cruz-Neto and Abé (1997) found that captive *G. soricina* reduced their body temperature when deprived of food. Facultative torpor has also been observed in other nectar-feeding bats, such as *L. curasoae* and *Choeronycteris mexicana* in captivity (C. Voigt, personal observation). Thus, some phyllostomids do reduce their body temperature under certain conditions. It could be argued that the low turnover rates in tissues of the two species of nectar-feeding bats could be due to prolonged periods of torpor, but two facts argue against this hypothesis. First, the bats of this study were always ready to fly from their roost when they were disturbed and torpid or lethargic bats are incapable of flight. Second, the nectar-uptake rate did not change during the course of the experiment, which would have been expected if bats used torpor after the switch in diets. Thus, torpor is an unlikely explanation for the observed low turnover rates.

Both *G. soricina* and *L. curasoae* lost approximately 8% of their initial body mass during the experiment. Nutritional deficits due to insufficient energy, mineral or nitrogen supplies, are possible causes for such a trend. Both species ingested approximately 20 ml of sugar water each day. As the bats were fed with 18% sugar water, this can be converted into a daily energy intake rate of approximately 60 kJ day$^{-1}$. This value falls into the range of previously reported rates of energy expenditure for both species (von Helversen and Winter, in press). In addition, Horner et al. (1998) estimated the field metabolic rate of *L. curasoae* as at least 40 kJ day$^{-1}$ and daily energy intake rates of non-reproductive *G. soricina* ranged from 7 to 68 kJ day$^{-1}$ in a captive study (Winter, 1993; Voigt, in press). We find it unlikely that the study bats suffered from an insufficient supply of energy. Other forms of nutritional stress may have occurred despite our intention to provide all essential nutrients such as vitamins and minerals.

In the present work, we simulated the extent to which fat mobilization affects the overall isotope ratio of the carbon pool that is available to the bat for homeostasis. In this simulation, we assumed that the isotopic fractionation between exogenous foods and tissues is the same as that for the metabolism of tissues and endogenous reserves. Assuming that (1) loss of body mass was solely due to the mobilization of fat and (2) body fat contained approximately 20% water, loss of 1.6 g body mass would be equivalent to approximately 1.3 g dry fat in a *L. curasoae* weighing 20 g. Assuming also that a

mammalian fat molecule consists of two $C_{16}$ chains and one $C_{14}$ chain, fat molecules would consist of 42 carbon, 100 hydrogen and 6 oxygen atoms. Taking the mass of carbon, hydrogen and oxygen into account, 1.3 g fat consists then of approximately 0.94 g carbon ($^{12}C$), 0.18 g hydrogen ($^{1}H$) and 0.18 g oxygen ($^{14}O$). Following this, 0.94 g carbon with an isotopic signature of approximately –22‰ $\delta^{13}C$ should have mixed with the ingested sugar molecules. *L. curasoae* ingested on average 20.4 ml sugar water per day, or 1.2 l sugar water during the 2-month period of the experiment. For reasons of simplicity, we have calculated all following values for glucose ($C_6H_{12}O_6$), which was the predominant sugar molecule in the diet. 1 ml of 18% sugar water contains 180 mg sugar. As nectarivorous bats absorb sugar in the intestine with an efficiency of almost 100% (Winter, 1998), each *Leptonycteris* is likely to have ingested 216 g sugar during the 2-month period. This is equivalent to approximately 86.4 g carbon, 14.5 g hydrogen and 115.1 g oxygen, respectively. Following this simulation, the ingestion of 216 g sugar results in the uptake of 86.4 g carbon from sugar ($\delta^{13}C$=–10‰), in contrast to 0.94 g carbon from fat ($\delta^{13}C$=–22‰). Thus, the overall isotope ratio of the carbon pool available for homeostasis would be –10.02‰, compared to –10.00‰ without fat mobilization. Even if fat was more depleted in heavy isotopes by approximately 3‰, a fractionation factor found in fat tissues of other animals, the overall $\delta^{13}C$ value would change only slightly to –10.05‰. Therefore, fat mobilization alone cannot explain the unexpectedly low estimates of isotope turnover rates seen when carbon isotopes are used for homeostasis, irrespective of their origin.

Preferential use of carbon from fat or proteins for tissue synthesis and carbon from sugar for energy synthesis could, however, change the above picture. Hobson and Stirling (1997) addressed this problem of selective metabolic pathways or differential isotopic routing. In a dietary study on polar bears, the authors found that carbon isotope ratios in blood were not distinguishable between offshore populations feeding on seals and inland populations feeding on berries, although seals as a fat- and protein-rich food source and berries as a carbohydrate-rich food source differed by approximately 9‰ in carbon isotope ratios. This led to the conclusion that inland populations probably burned the carbohydrates from berries directly instead of incorporating them into tissues. This is a likely explanation in the specific case. In contrast to polar bears, nectar-feeding bats are dietary specialists depending strongly and sometimes even exclusively on nectar as their food source. Bats may supplement their diet with pollen, fruits and to some extent with insects, but the main carbon source of nectar-feeding bats is nectar. Therefore, nectar-feeding bats ultimately have to incorporate nectar carbon into their tissue. However, it is likely that in our study that carbon isotopes from mobilized body substrates were incorporated into the blood, thus mixing with the carbon of assimilated sugar. Obviously, this effect should be most apparent in starving animals and absent in animals with constant body mass. Hatch et al. (1995) showed that the catabolic states of rooster chicks and adult hens are indicated by increased levels of $\delta^{13}C$ in haemoglobin. In both species of our study, we find individuals that lost body mass and individuals that maintained an almost constant body mass during the experiment (Fig. 2). We expected that the regression coefficient *c* of the estimated exchange curve would be underestimated in catabolizing animals and that the coefficient should reflect true values in individuals with constant body mass. We tested for a significant positive relationship between the regression coefficients *c* and body mass loss in four tissues, but found only a negative correlation for *G. soricina* blood (Fig. 4). Therefore, loss of body mass did not result in an overestimate of the isotope turnover half life in these specific cases. Possibly, additional fractionation effects obscured the presumed positive correlation between loss of body mass and regression coefficient, or may even have reversed this trend.

Bat blood is unusual in several ways. First, blood from bats contains more erythrocytes per ml than in other mammals ($26\times10^6$ erythrocytes $ml^{-1}$ blood in bats *versus* approximately $18\times10^6$ erythrocytes $ml^{-1}$ blood in Rodentia or Insectivora; summarized by Neuweiler, 1993). Secondly, hemoglobin concentrations are higher in bats than in other mammals or even birds (0.24 g $ml^{-1}$ blood in a pipistrelle bat versus 0.18 g $ml^{-1}$ blood in hummingbirds; summarized by Neuweiler, 1993). These extreme values might be an adaptation to the high energy demands of flight (Voigt and Winter, 1999; Voigt, 2000). In birds, the oxygen capacity of blood is maximized by unidirectional lungs, resulting in an optimal oxygen uptake. Assuming that similar sized bats and birds are producing erythrocytes at similar rates, we hypothesize that bats reach a similar oxygen capacity of blood by having larger amounts of smaller, longer-lived erythrocytes. Such a mechanism could compensate for the less efficient bidirectional lung of bats. Thus, long-lived erythrocytes may be an adaptation of bats to the high energy demands of flight. By using the same experimental setup and separating the cellular fraction of blood from plasma by centrifugation (see also Hobson and Clark, 1993), it will be possible to test this idea.

In summary, the estimated turnover rates of isotopes in the target tissues were consistently low for both study species. In *L. curasoae*, we could trace small amounts of carbon isotopes from $C_4$-sugar in the hair, but no significant amounts from $C_4$-sugar could be found in hair of *G. soricina*. This finding is probably explained by the seasonal growth of hair. The rate of isotope turnover in wing membrane was low, with $t_{50}$ values ranging from 100 to 130 days. The particular composition and design of the wing membrane and its relatively low temperature are likely explanations for this result. In blood, the turnover rate of carbon isotopes was unexpectedly low. The carbon isotope half life was 120–126 days. We suggest that long-lived erythroycytes are a special adaptation of bats for maintaining a high oxygen capacity of blood, which is a prerequisite for enduring, aerobic flight performance. Further investigations are needed in the field of bat blood physiology.

We wish to express our thanks to Christiane Stemmer for preparing the samples for isotope analysis and to Michael Joachimski (stable isotope laboratory, department of geology, University of Erlangen-Nürnberg, Germany) for analysing part of the samples. Many thanks go to Otto von Helversen for allowing us to work with the bats and to Monika Otter for maintaining the captive colony. We thank Heribert Hofer, Roland Frey and two anonymous reviewers for valuable discussion.

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