The Journal of Experimental Biology 207, 1741-1748
Published by The Company of Biologists 2004
doi:10.1242/jeb.00929

Nitrogen stress causes unpredictable enrichments of ^{15}N in two nectar-feeding bat species

Christian C. Voigt[1,*] and Felix Matt[2]

[1]*Institute for Zoo and Wildlife Research, Evolutionary Ecology Research Group, Postfach 601103, 10252 Berlin, Germany* and [2]*Institute of Zoology II, University Erlangen-Nürnberg, Staudtstr. 5, 91058 Erlangen, Germany*

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

Accepted 6 February 2004

Summary

We estimated the effect of nitrogen stress on the nitrogen isotope enrichments in wing membrane and blood of two nectar-feeding bats (*Glossophaga soricina* and *Leptonycteris curasoae*) by offering a nitrogen-poor diet with a high $\delta^{15}N$ and $\delta^{13}C$. Before the experiment, bats were sustained on a normal diet with a low $\delta^{15}N$ and $\delta^{13}C$. Under this first food regime, the fractionation of nitrogen isotopes averaged 3.1‰ $\delta^{15}N$ for blood and 4.4‰ $\delta^{15}N$ for wing membrane, which was almost twice as high as the corresponding fractionation of carbon isotopes. After switching to the nitrogen-poor diet, the enrichment of heavy isotopes increased for both elements in all tissues under study. The recently published estimates of half-life of carbon isotopes indicated a low turnover rate of carbon in wing membrane and blood and an almost constant half-life over varying losses of body mass. The estimates of half-life of nitrogen were two to six times higher than those of carbon. We argue that this discrepancy was caused by the mixing of nitrogen isotopes from internal and external sources. The mixing effect was probably negligible for carbon as the amount of ingested carbon outweighed the amount of mobilized carbon from internal sources. A correlation between the estimated turnover rates of nitrogen and losses of body masses was probably obscured by the additional fractionation of nitrogen isotopes in catabolic animals. We conclude that the interpretation of nitrogen isotope data of free-ranging animals is difficult when the animal's diet is changing to a critical nitrogen content.

Key words: nitrogen isotope, nitrogen stress, fractionation, mixing, *Glossophaga soricina*, *Leptonycteris curasoae*.

Introduction

Since the landmark publications by DeNiro and Epstein (1978, 1981), stable isotopes have been increasingly used in ecophysiological studies. The authors were among the first to point out the observation that the carbon and nitrogen isotope ratios in animal tissues are correlated with the isotopic composition of the animal's diet. This observation was confirmed for a wide variety of animals (e.g. Cabana and Rasmussen, 1994; Minagawa and Wada, 1984; Owens, 1987; Peterson and Fry, 1987) and was commonly translated into the phrase "*you are what you eat…plus a few per mil*". During the past few years, various researchers have used isotopic data of various elements to trace diets (e.g. Ben-David et al., 1997; Hobson et al., 2000), trophic relationships (Eggers and Jones, 2000; Hobson and Welch, 1992) or migratory movements of animals (e.g. Fleming et al., 1993; Hobson, 1999).

An important assumption in stable isotope studies is the premise that tissue isotope ratios equal dietary isotope ratios plus an offset called fractionation factor or the consumer-diet enrichment (DeNiro and Epstein, 1978, 1981; Mizutani et al., 1992). Fractionation describes the phenomenon that light and heavy isotopes pass through the body at different rates due to enzymatic and physical processes; heavy isotopes are preferentially held back in the body, whereas light isotopes are preferentially released. The isotopic analysis of whole animals shows, for example, an enrichment of ^{15}N by 3.0±2.6‰ $\delta^{15}N$ relative to the diet (DeNiro and Epstein, 1981). Within animals, tissues differ in their fractionation of isotopes according to their molecular composition and the specific enzymes involved. In muscles of birds, diet-tissue enrichments of ^{15}N vary between 0.2‰ and 1.4‰ $\delta^{15}N$ (Hobson and Clark, 1992) and in feathers between 2‰ and 6‰ $\delta^{15}N$ (Hobson and Clark, 1992; Mizutani et al., 1992). Fractionation effects also occur in other animal groups, such as mammals or insects, and within all groups fractionation factors vary between species and tissues (e.g. DeNiro and Epstein, 1981; Hilderbrand et al., 1996; Webb et al., 1998).

Catabolic animals are more enriched in heavy nitrogen isotopes than animals with a balanced energy budget (Hobson and Clark, 1992; Hobson et al., 1993). In the remainder of this text, we call this phenomenon 'additional fractionation', because an additional offset leads to a larger difference in isotope ratios between diet and tissue than in animals with a balanced energy budget. Recently, Vanderklift and Ponsard (2003) discussed the effect of poor-quality diets (i.e. with a

high C:N ratio) on the ^{15}N enrichments of animals and the overall lack of experiments. In the present study, we investigated the effect of nitrogen stress on ^{15}N enrichments in two nectar-feeding bat species, i.e. we studied whether estimates of tissue turnover based on nitrogen isotopes are reliable in animals under nitrogen stress. In an earlier paper, we described the fractionation and half-life of carbon isotopes in an experiment with two nectar-feeding bat species: *Leptonycteris curasoae* (20 g) and *Glossophaga soricina* (10 g) (Voigt et al., 2003b). We switched the carbon isotope ratio of the diet by approximately 14‰ $\delta^{15}N$ and traced the change in carbon isotope enrichments in different tissues (wing membrane, blood and hair) over a period of 60 days. We observed that the half-life of stable carbon isotopes was consistently low for both species and all tissues. In contrast to our expectation, we could not find a negative correlation between half-life of carbon isotopes and losses of body mass. We concluded that the mobilization of body reserves and associated mixing effects did not bias the estimates of tissue turnover of carbon isotopes because the amount of carbon from internal sources was negligible in relation to the amount of carbon from external sources.

During the same experiment, the nitrogen concentration of the diet was changed from ~1.3% to 0.1% nitrogen, and the nitrogen isotopic ratio from 4.2‰ to 13.1‰. Here, we present and discuss the results of this aspect of the experiment. For wing membrane and blood, we predicted that $\delta^{15}N$ would increase due to the replacement of light with heavy nitrogen isotopes from external sources. Secondly, we predicted that the loss of body mass would lead to an overestimate of half-life because light nitrogen isotopes from internal sources (body substance) mix with heavy nitrogen isotopes from the diet. Therefore, the half-life of nitrogen isotopes should be negatively correlated with losses of body mass. Thirdly, we predicted that carbon and nitrogen isotope turnover rates would be correlated within the same tissue if additional fractionation is absent.

Materials and methods

Details of the experiment are described in Voigt et al. (2003b). We transferred 10 *Glossophaga soricina* Pallas (three males and seven non-reproductive females) and 10 *Leptonycteris curasoae* Miller (seven males and three non-reproductive females) (Phyllostomidae: Glossophaginae) from a captive breeding colony maintained in greenhouse facilities at the University of Erlangen-Nürnberg into two indoor flight enclosures (4 m×3 m×3.5 m). For individual identification, we banded all bats with coloured and numbered plastic bands on the forearm (size XL for *L. curasoae* and XCL for *G. soricina*; A.C. Hughes, Hampton Hill, UK). The two groups were maintained over a period of 70 days with food provided *ad libitum* at three artificial feeders. Room temperature was set constant to approximately 23°C, the ambient humidity to 70% and the light:dark regime to 12 h:12 h. The bats were habituated to the enclosure over a one-week period and fed with the same diet as in the breeding colony. This food originated from plants representative of the C_3 photosynthetic pathway and a low $\delta^{15}N$ value (Table 1). The different types of diet were diluted in water to equal 18% sugar water (mass/mass, refractometer, accuracy 0.2%).

After seven days of habituation, we switched the diet of the bats to plants representative of the carbon isotope composition of C_4 and CAM photosynthetic pathways and a high $\delta^{15}N$ value (Table 1). We refer to this day as day one of the experiment. As before, all different food sources were mixed to a sugar concentration of 18% (mass/mass). To complement the diet of the bats, we added several mg of vitamins and minerals to the diet each day.

Before and after each night, the sugar water was weighed to an accuracy of 1 g and we refer to the ingested food as the difference between the two measurements. The overall nitrogen isotope enrichment of the ingested food was estimated each day by measuring the portion of each of the three food sources ingested by the animals by the percentage concentration of nitrogen in the dry substance of the food (Tables 1, 2) and by multiplying these values by the corresponding $\delta^{15}N$ (Tables 1, 2). To estimate the mean isotopic enrichment of the ingested food before and after switching the diet, we calculated the average value of isotopic enrichment of the food for seven days *before* day one and for 60 days *following* day one. Then, we calculated mean values for the period before day one and following day one. The change in isotopic composition of diets equalled 8.9‰ $\delta^{15}N$ for both species (*L. curasoae*: diet 1=4.1‰ $\delta^{15}N$, diet 2=13.0‰ $\delta^{15}N$; *G. soricina*: diet 1=4.3‰ $\delta^{15}N$, diet 2=13.2‰ $\delta^{15}N$).

At the end of the first week and during each subsequent

Table 1. *Nitrogen isotopic composition of the diet before (diet 1) and during (diet 2) the experiment*

	Type	% Nitrogen (mean ± S.D.)	$\delta^{15}N$ (‰) (mean ± S.D.)
Diet 1	Alete	2.35[a]	6.49[a]
	Honey	[b]	[b]
	Nektar Plus	0.31±0.12	–0.85±0.68
	Pollen	*3.20±0.42*	*1.84±1.67*
	Banana	*0.53*[a]	*–0.04*[a]
Diet 2	Cane sugar	0.07[a]	13.54[a]
	Corn syrup	0.07±0.02	10.30±2.85
	Agave syrup	[b]	[b]
	Opuntia fruits	*0.71±0.03*	*5.47±0.05*

[a]Single measurement; [b]amounts of N were insufficient for measurements.

Alete, honey and Nektar Plus were the three main food sources during the initial week prior to the diet switch. In diet 1, food was supplemented by small amounts of pollen and banana. Cane sugar, corn syrup and agave syrup were the three main food sources starting from day one of the experiment. In diet 2, food was supplemented by small amounts of opuntia fruits. Minor food sources are in italic.

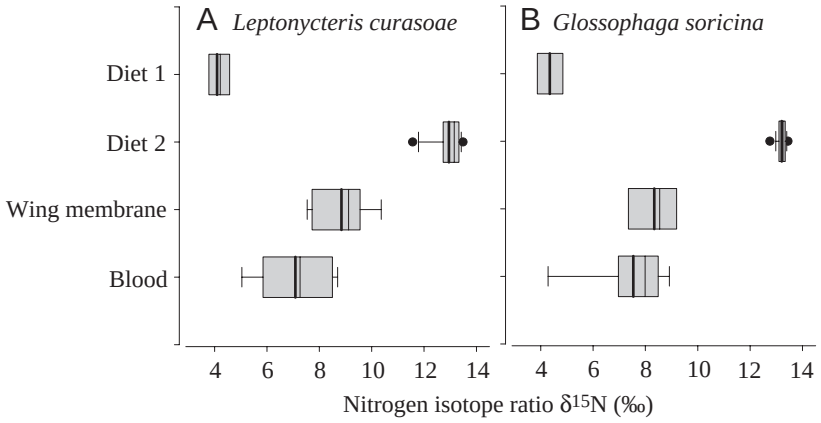

Fig. 1. Nitrogen isotope ratios ($\delta^{15}N$; ‰) of the two diets and in wing membrane and blood of *Leptonycteris curasoae* (A) and *Glossophaga soricina* (B) equilibrated to diet 1. Data are given as box plots with the border of the boxes representing the 25 and 75 percentiles, the T-mark the 10 and 90 percentiles and the outermost points the 5 and 95 percentiles. The thick line within the box indicates the mean, and the thin line indicates the median.

sampling event (in two-week intervals), bats were weighed to an accuracy of 0.01 g (electronic balance, Mettler PM-100) and two types of samples were taken from each bat. We took two tissue samples from the wing membrane using a sterile 3 mm-diameter biopsy punch. Next, we drew approximately 30 µl of blood from the animals by puncturing the propatagial vein with a small sterile needle. 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 refrigerator below 0°C. After the change in diets, we captured the bats and took ~30 µl blood and a small piece of wing membrane at the end of the second, fourth, sixth and eighth week. To remove external contaminants from the skin, we washed the samples with a chloroform:methanol solvent (1:1; Voigt et al., 2003b).

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 (Bremen, Germany). The sample isotope ratios were compared with international gas standards (USGS-24 and IAEA-N1). Accuracy was greater than ±0.1‰ (1σ). Isotope ratios are expressed in the δ notation in parts per thousands (‰) using the following equation:

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

We used the nitrogen isotope ratio of the atmosphere as the standard.

Statistical analysis and curve estimation

To test for differences in mean isotope enrichments between tissues, we performed a one-way analysis of variance (ANOVA). We ran *post-hoc* Tukey HSD tests to make pair-wise comparisons to evaluate differences in mean values. We calculated mean values of isotopic composition 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 $y=a+b\times e^{c\times t}$ were fitted to the $\delta^{15}N$ data from each tissue and each bat species. In this equation, a represents the asymptotic $\delta^{15}N$ 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 nitrogen isotopes in the tissues, and y is the mean nitrogen isotope ratio in the tissue at time t. For 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 that is equal to the baseline value at day one plus the difference in isotope enrichment between the two diets. Estimation of c was performed on an iterative basis starting with a value of 0.05. To estimate the half-life of nitrogen isotopes, we calculated t_{50} using the following equation: $t_{50}=\log_e(0.5)/c$, with t_{50} representing the time in days in which half of the nitrogen isotopes were exchanged in the corresponding tissue, $\log_e$ representing the natural logarithm and 0.5 representing the exchange of 50% isotopes. Some tissue and blood samples contained amounts of nitrogen that were insufficient for mass spectrometer analysis. Therefore, we excluded the estimated regression coefficients of these individuals from further analysis (one blood and wing membrane sample of *L. curasoae* and two blood and wing membrane samples of *G. soricina*).

Possible effect of body mass changes on regression coefficients

To evaluate a possible influence of the loss of body mass on the estimates of t_{50}, we calculated exponential exchange curves for nitrogen isotopes on an individual basis. We then tested whether the half-life of nitrogen in individual tissues was correlated with the change in body mass. We predicted that the half-life of nitrogen isotopes decreases with loss of body mass. The level of significance was Bonferroni-corrected to 2.5%, because two data sets were tested in each individual. Mean values ± 1 S.D. are expressed. Unless stated otherwise, two-tailed tests were performed. For all statistical analysis and regression models we used SPSS (version 9.0).

Results

The difference in ^{15}N enrichment between the initial diet and blood equalled 3.0‰ in *L. curasoae* and 3.2‰ in *G. soricina* (Fig. 1). Wing membrane was even more enriched in ^{15}N than

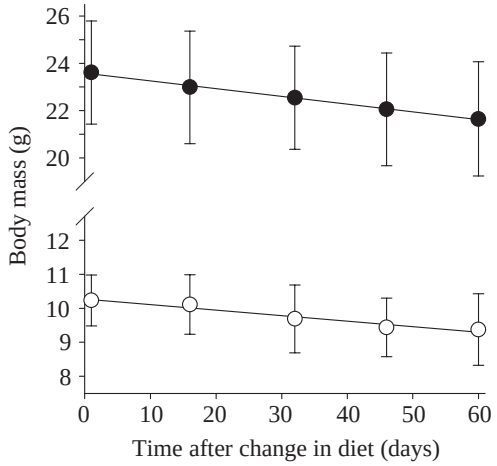

Fig. 2. Mean changes in body mass (±1 S.D.) of *Leptonycteris curasoae* (solid circles) and *Glossophaga soricina* (open circles) during the experiment. Linear regressions were calculated over mean values for each species (solid lines). On average, both species lost 8% of their body mass during the experiment.

the diet by 4.7‰ in *L. curasoae* and 4.0‰ in *G. soricina*. The differences in enrichment of ^{15}N between tissues were significant for *L. curasoae* (one-way ANOVA: $F_{1,18}$=10, P=0.006) but not for *G. soricina* ($F_{1,18}$=1.55, P=0.23).

Data on nutritional intake are reported in Voigt et al. (2003b) and repeated briefly here for reasons of completeness. The nutritional intake of the bats remained constant throughout the experiment. On average, individuals of *L. curasoae* ingested 20.3±2.9 ml day^{-1} and those of *G. soricina* ingested 19.2±1.8 ml day^{-1}. The mean initial body mass equalled 23.6±2.1 g in *L. curasoae* and 10.2±0.7 g in *G. soricina* (Fig. 2). Both species lost on average 8% of their initial body mass during the course of the experiment.

After switching the diet to plant products with higher $\delta^{15}N$ values, the enrichment of ^{15}N increased significantly in both tissues (Fig. 3). At the end of the experiment at day 60, none of the tissues was equilibrated to the expected point of nitrogen isotopic enrichment (first numerical in the exponential regression equation plotted in the graphs). The nitrogen isotope turnover was estimated as 821 days in wing membrane and 514 days in blood for *L. curasoae*, or 329 days in wing tissue and 274 days in blood for *G. soricina* (see Fig. 3). Estimates of t_{50} that were based on nitrogen isotopes were significantly higher than estimates that were based on carbon isotopes (Wilcoxon signed-rank test *L. curasoae* blood, T=0, N=10, P<0.05; wing membrane, T=7, N=10, P<0.05; *G. soricina* blood, T=3, N=10, P<0.05; wing membrane, T=0, N=10, P<0.05; carbon isotope data from Voigt et al., 2003b). According to Levene's test, the variances of estimated half-lifes were significantly higher for nitrogen isotopes than for carbon isotopes (*L. curasoae* blood $F_{1,18}$=15.2, P=0.001; wing membrane $F_{1,18}$=6.5, P=0.02; *G. soricina* blood $F_{1,18}$=14.0, P<0.001; wing membrane $F_{1,18}$=9.1, P<0.007; carbon isotope data from Voigt et al., 2003b). The regression coefficients of nitrogen exchange curves of individual bats and consequently also the t_{50} values were not affected by body mass losses (Fig. 4; Table 2). Additionally, within the same tissue of the same individual, the corresponding pairs of turnover rates and t_{50} values, respectively, were not significantly correlated with each other (Table 3).

Table 2. *Spearman rank correlation coefficients for the relationship between* t_{50} *based on nitrogen isotopes and loss of body mass in* Leptonycteris curasoae *(LC) and* Glossophaga soricina *(GS)*

Sample type	r_S	P
Blood LC	–0.42	0.30
Wing membrane LC	–0.19	0.62
Blood GS	0.20	0.30
Wing membrane GS	0.02	0.83

Neither in *L. curasoae* nor in *G. soricina* was the estimated t_{50} value significantly related to the loss of body mass.

Table 3. *Spearman rank correlation coefficients for the relationship between* t_{50} *based on carbon isotopes and* t_{50} *based on nitrogen isotopes in* Leptonycteris curasoae *(LC) and* Glossophaga soricina *(GS)*

Sample type	r_S	P
Blood LC	0.18	0.63
Wing membrane LC	0.25	0.48
Blood GS	–0.06	0.88
Wing membrane GS	0.04	0.93

Neither in *L. curasoae* nor in *G. soricina* were the two t_{50} values derived for the same tissue significantly correlated.

Discussion

Enzymatic and physical processes differentiate between isotopes of an element and this leads to an enrichment of heavy isotopes in organisms relative to their diet (e.g. DeNiro and Epstein, 1978, 1981; review in Vanderklift and Ponsard, 2003). In the present study, the enrichment of ^{15}N between diet and wing membrane or diet and blood ranged from 3.0‰ to 4.7‰, and samples from wing membrane were more enriched in ^{15}N than were samples from blood. The average fractionation of nitrogen isotopes in bat blood equalled 3.1‰, which was almost identical to the average value described for mammals (~3.0‰ in Vanderklift and Ponsard, 2003). The enrichment of nitrogen isotopes between wing membrane and diet averaged 4.4‰ for the two study species, and no data are currently available for comparison. Compared with the average fractionation of carbon isotopes in the same tissue, ^{15}N was enriched twice as much as ^{13}C in the same tissue (Voigt et al., 2003b). This is consistent with findings of other authors who report a twofold difference in the fractionation

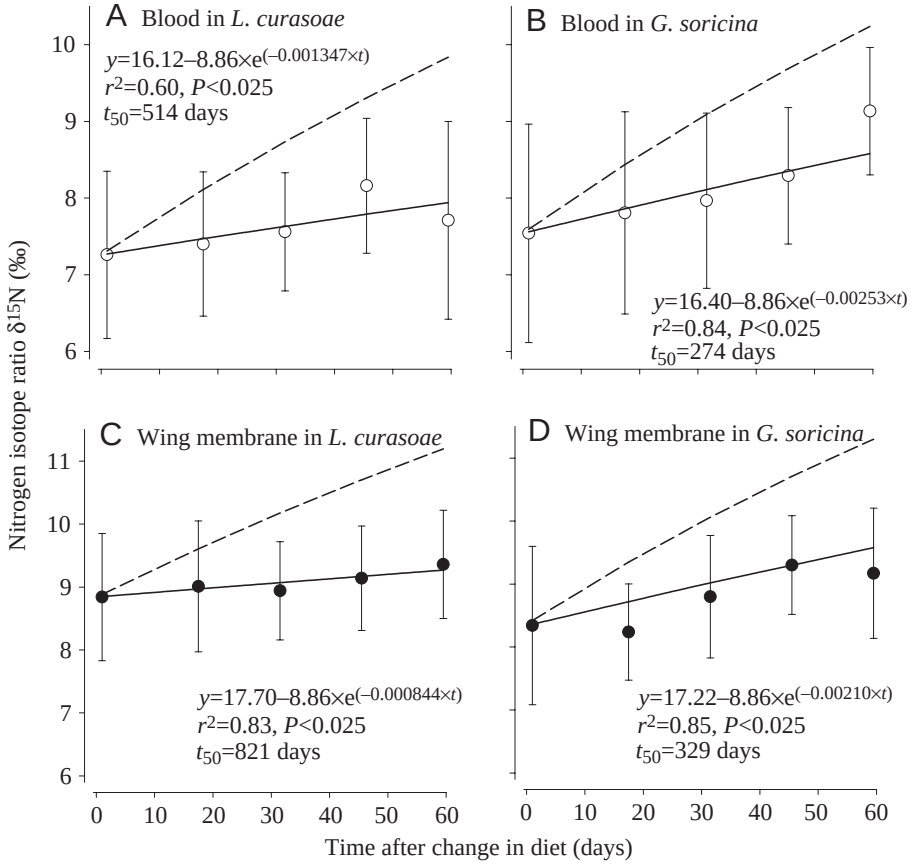

Fig. 3. Changes in nitrogen isotope ratio ($\delta^{15}N$; ‰) in two tissues of *Leptonycteris curasoae* (A,C) and *Glossophaga soricina* (B,D) after the diet was changed at day one to plant products with a nitrogen isotope ratio that was more enriched in ^{15}N by ~9‰ $\delta^{15}N$ than the initial diet; mean values (± 1 S.D.) for blood are shown in A and B (open circles) and those for wing membrane in C and D (filled circles). Exponential regression functions were fitted to the data sets (solid line; see equations in the corresponding graphs). Broken lines give the expected regression lines calculated with the corresponding regression coefficients derived from the carbon isotope data set (Voigt et al., 2003b).

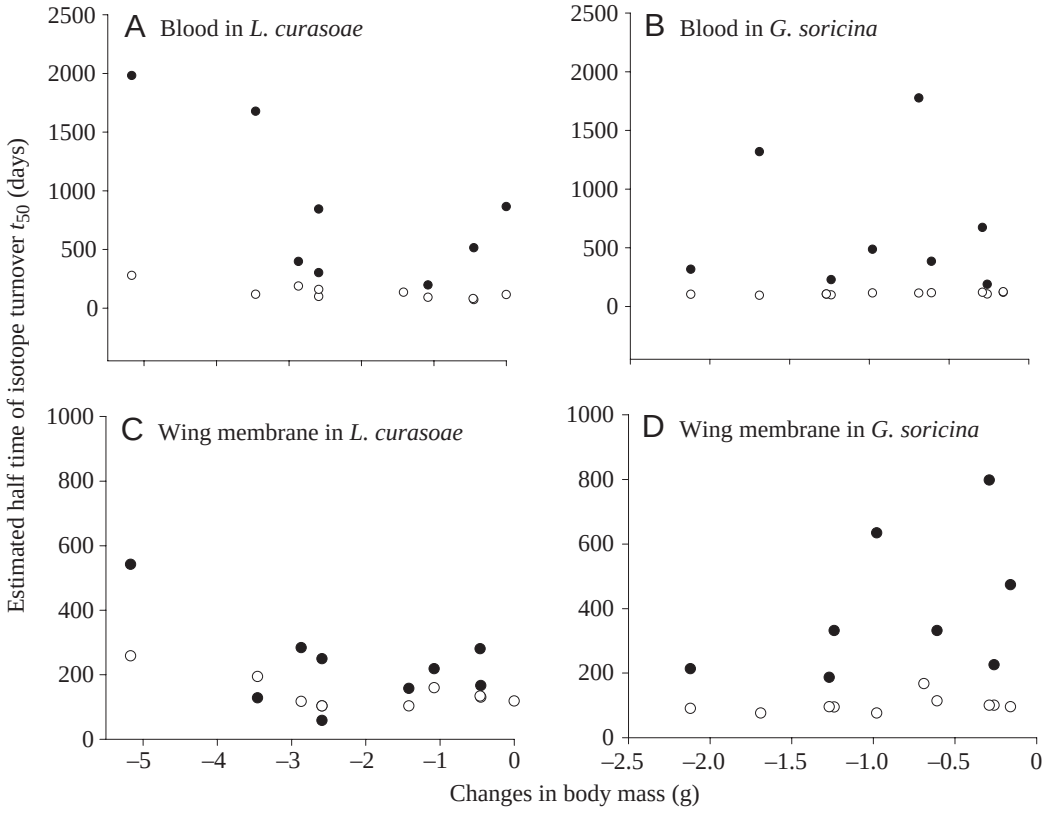

Fig. 4. Relationship between the estimated half-life of isotopes in blood (A,B) and wing membrane (C,D) (filled circles, nitrogen isotopes; open circles, carbon isotopes; data from Voigt et al., 2003b). The estimated half-life of nitrogen isotopes was not significantly correlated with body mass loss (Table 2). The sample volume was too small in some cases to measure the nitrogen isotope ratio. Thus, we could not calculate individual t_{50} values for these individuals (one data point in A and C; two data points in B and D).

effect of carbon and nitrogen isotopes (e.g. Mizutani et al., 1992).

During the experiment, individuals of both species ingested approximately 19 ml sugar water each day, which is similar to their daily food intake in similar experimental setups (e.g. Voigt, 2003; Voigt et al., 2003a). The amount of ingested sugar water did not change over time (Voigt et al., 2003b). Both facts argue against a situation of energetic malnutrition. Nonetheless, almost all bats lost body mass during the experiment, and this loss of body mass could be caused by an insufficient supply of nitrogen (Tables 1, 2). Our previous study showed that the estimates of half-life based on carbon isotopes remained almost constant irrespective of the amount of mobilized body substance (Fig. 4; Voigt et al., 2003b). This lack of correlation is probably due to the fact that (1) the amount of carbon from internal sources, i.e. body substance, is negligible compared with that from dietary sources and (2) fractionation effects are less pronounced in carbon isotopes. The larger portion of nitrogen from internal sources in relation to external sources from a nitrogen-poor diet (Table 1) may, however, influence the estimates of nitrogen half-life. In the following paragraph, we will simulate the extent of this effect.

Howell (1974) estimated a protein requirement of 84–106 mg protein day^{-1} for *L. curasoae*. Dividing this value by 6.25 (a standard factor for the conversion of protein to nitrogen; Kleiber, 1961) yields a nitrogen requirement of 13–17 mg nitrogen day^{-1} for *L. curasoae*. The percentage concentration of nitrogen in the second diet was low (mean 0.07%), but even lower values were found in the nectar of bat- or bird-pollinated plants; for example, Baker and Baker (1982) measured a mean protein content of 0.04% in dry matter, which is equivalent to 0.0064% nitrogen in dry nectar. *L. curasoae* ingested on average 20 ml day^{-1} of 18% (mass/mass) sugar water or 3.6 g day^{-1} pure sugar. This is equivalent to an average daily nitrogen uptake of 3.6 g×0.07%=2.52 mg nitrogen from sugar water. In addition to the dietary nitrogen uptake, *L. curasoae* also acquired nitrogen from body substance. We consider two scenarios: (1) all loss of body mass is due to fat mobilization and (2) all loss of body mass is due to protein catabolism.

Body fat contains ~20% water and therefore mobilization of 1.9 g body mass in 60 days equals 1.5 g fat. According to Kleiber (1961), dry fat consists of 7% protein or 7/6.25=1.1% nitrogen. Mobilization of 1.5 g fat released 1.5 g×0.011%=0.0165 g nitrogen in 60 days, or 0.28 mg nitrogen day^{-1}. Thus, individual *L. curasoae* had a total amount of 2.82 mg nitrogen available per day: 0.3 mg nitrogen from fat mobilization and 2.52 mg nitrogen from sugar water.

Protein consists of 16% nitrogen (Kleiber, 1961). As non-fat body substance consists of 70% water in nectar-feeding bats (Voigt et al., 2003a), *L. curasoae* catabolized ~0.38 g of protein dry matter in 60 days. According to this, bats mobilized 0.0608 g nitrogen in 60 days, which is equivalent to 1.0 mg nitrogen day^{-1}. Under the second scenario, *L. curasoae* had a total amount of 3.52 mg nitrogen available per day: 1.0 mg nitrogen from protein catabolism and 2.52 mg from sugar water. Both simulations do not take into account that bats supplemented their diet with small amounts of nitrogen-rich opuntia fruits. Therefore, the estimates of available nitrogen are probably underestimated. Nonetheless, does this simulation emphasize that (1) the nitrogen requirements of the bats (~13–17 mg day^{-1}; Howell, 1974) were not met during the experiment although mobilized body reserves provided additional nitrogen and that (2) more nitrogen was available from external sources than from internal sources.

In the present study, the mobilization of body reserves may have influenced the isotopic enrichments within tissues, because nitrogen isotopes of body reserves (internal substrate) with a low $\delta^{15}N$ ratio mixed with those of the diet (external substrate). If both isotope pools are combined to intermediate levels of $\delta^{15}N$, the half-life (t_{50}) is overestimated. In agreement with this, t_{50} estimates were higher than the values based on carbon isotopes (Figs 3, 4). The estimates of blood half-life derived from carbon isotopes equalled 120 days in *L. curasoae* and 113 days in *G. soricina*, and the estimates for wing membrane equalled 134 days in *L. curasoae* and 102 days in *G. soricina*. Comparing these values with those of Fig. 3 reveals that the estimates of turnover rates based on nitrogen isotopes were, on average, two to six times higher. Interestingly, the estimates of half-life based on nitrogen isotopes (Fig. 3) were approximately twice as high in *L. curasoae* than in *G. soricina*: 1.8 times in blood (514/274) and 2.5 times in wing tissue (821/329). This factor of two could be explained by the observation that *L. curasoae* mobilized approximately twice as much body reserves as did *G. soricina* (1.9 g in *L. curasoae* and 0.8 g *G. soricina*), while ingesting approximately the same amount of sugar water as *G. soricina* (~19 ml day^{-1} in both species). Following this, the ratio between external and internal nitrogen pools differed by a factor of two between the study species; in other words, *L. curasoae* derived twice as much nitrogen isotopes from internal sources than did *G. soricina*. We conclude that mixing effects of nitrogen isotopes occurred during the experiment. In summary, estimates of half-life based on nitrogen isotopes were most likely influenced by mixing effects, whereas those based on carbon isotopes were not influenced by loss of body mass because the amount of mobilized carbon from internal sources was small compared with the amount of carbon from external sources (Voigt et al., 2003b).

In contrast to our expectation, the estimates of t_{50} that were based on nitrogen isotopes did not correlate with losses of body mass (Table 2). A possible explanation for this finding is that simultaneous fractionation effects, as described for example in birds (Hatch et al., 1995), obscured the true turnover rates. Several authors have suggested that the fractionation of nitrogen isotopes most likely occurs during the deamination and transamination of amino acids (e.g. Gaebler et al., 1966; Minagawa and Wada, 1984). Enzymatic processes necessary for homeostasis may then lead to even higher enrichments of heavy nitrogen within tissues. Hobson and Clark (1992) proposed that "*nutritional stress cause[d] substantial increases in diet-tissue fractionation values due either to: (1) mobilization and*

redeposition of proteins elsewhere in the body; or (2) amino acid composition changes in tissues". Following this argument, one could expect $\delta^{15}N$ in mobilized organic molecules to be higher in comparison with the initial state of the organic molecules in the body reserves. Hatch et al. (1995), for example, found that chicks kept under a restricted food regime grew less and had higher $\delta^{13}C$ values in tissues than chicks fed *ad libitum*. This effect of additional fractionation should be even more pronounced in nitrogen isotopes because nitrogen isotopes fractionate to a larger extent than carbon isotopes (present study; Mizutani et al., 1992). Hobson et al. (1993) observed high enrichments of ^{15}N in tissues of Ross's geese (*Chen rossii*) that had been fasting for four weeks. Similar results were obtained in Japanese quails (*Coturnix japonica*) when raised on a restricted diet (Hobson et al., 1993). A second possible explanation is that the different extent of metabolic processing of two nitrogen sources, i.e. internal body reserves (fat) and external food sources (carbohydrates), may lead to different nitrogen enrichments because the enzymes involved in the catabolism of fat and carbohydrates are active to different degrees and because these enzymes may fractionate nitrogen isotopes at different rates. This hypothesis could also explain the well-known phenomenon in feeding trials that the same organism shows different enrichments in ^{15}N when fed on diets of different quality (e.g. Webb et al., 1998; Adams and Sterner, 2000; Oelberman and Scheu, 2002); i.e. the assimilation of diets of different composition requires corresponding enzymes with specific fractionation characteristics, which in turn results in diet-specific enrichments of ^{15}N in the tissue of the organism. We argue in the present study that bats complemented their nitrogen requirements by recycling nitrogen from catabolized body substance. Thus, complex fractionation effects, in addition to the mixing of nitrogen isotopes from internal and external pools, led to the observed overestimate of t_{50} and the large variance of t_{50} values (see Fig. 4). In addition, individual differences in metabolic rates could have attributed to the variance in t_{50} estimates, as enzymatic action correlates with the metabolism of an animal.

In summary, half-lifes of blood and wing membrane estimated using nitrogen isotopes were higher than those estimated using stable carbon isotopes when bats were sustained under a nitrogen-poor diet. In addition, isotope turnover rates estimated with carbon and nitrogen isotopes were not correlated. These findings are most likely explained by mixing effects, additional fractionation of nitrogen isotopes and individual metabolic rates. The present study shows that nitrogen stress and the associated mobilization of body substance or compensatory metabolic responses may alter the isotopic composition of tissues. This could violate two basic assumptions of stable isotope studies: (1) isotopic balance within tissues and (2) constant and predictable fractionation factors between diet and tissues. Therefore, seasonal changes in the diet, for example, may be difficult to track with nitrogen isotopes if diet quality and body masses change seasonally as well, e.g. in reproducing, migrating or hibernating animals.

We wish to express our thanks to Christiane Stemmer for preparing the samples for isotope analysis and to Dr Michael Joachimski for the analysis in the Stable Isotope Laboratory of the Department of Geology at the University of Erlangen-Nürnberg. We thank Otto von Helversen for permission to use his facilities and to work with the captive colony of bats. We also thank Monika Otter for maintaining the captive colony of bats. We are grateful to Sylvia Ortmann and Katja Rex for valuable discussion. This study was partly financed by the Deutsche Forschungsgemeinschaft (FOR 402).

References

Adams, S. A. and Sterner, R. W. (2000). The effect of dietary nitrogen content on trophic level ^{15}N enrichment. *Limn. Oceanogr.* **45**, 601-607.

Baker, H. G. and Baker, I. (1982). Chemical constituents of nectar in relation to pollination mechanisms and phylogeny. In *Biochemical Aspects of Evolutionary Biology* (ed. H. M. Nitecki), pp. 131-171. Chicago: Chicago University Press.

Ben-David, M., Flynn, R. W. and Schell, D. M. (1997). Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. *Oecologia* **111**, 280-291.

Cabana, G. and Rasmussen, J. B. (1994). Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* **372**, 255-257.

DeNiro, M. J. and Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **42**, 495-506.

DeNiro, M. J. and Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* **45**, 341-351.

Eggers, T. and Jones, T. H. (2000). You are what you eat … or are you? *Trends Ecol. Evol.* **15**, 265-266.

Fleming, T. H., Nunez, R. A. and Sternberg, L. (1993). Seasonal changes in the diets of migrant and non-migrant nectarivorous bats as revealed by carbon stable isotope analysis. *Oecologia* **94**, 72-75.

Gaebler, O. H., Vitti, T. G. and Vukmirovich, R. (1966). Isotope effects in metabolism of ^{14}N and ^{15}N from unlabelled dietary proteins. *Can. J. Biochem.* **44**, 1249-1257.

Hatch, K. A., Sacksteder, K. A., Treichel, I. W., Cook, M. E. and Porter, W. P. (1995). Early detection of catabolic state via change in $^{13}C/^{12}C$ ratios of blood proteins. *Biochem. Biophys. Res. Commun.* **212**, 719-726.

Hilderbrand, G. V., Farley, S. D., Robbins, C. T., Hanley, T. A., Titus, K. and Servehen, C. (1996). Use of stable isotopes to determine diets of living and extinct bears. *Can. J. Zool.* **74**, 2080-2088.

Hobson, K. A. (1999). Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* **120**, 314-326.

Hobson, K. A. and Clark, R. G. (1992). Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. *Condor* **94**, 189-197.

Hobson, K. A. and Welch, H. E. (1992). Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. *Mar. Ecol. Prog. Ser.* **84**, 9-18.

Hobson, K. A., Alisauskas, R. T. and Clark, R. G. (1993). Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. *Condor* **95**, 388-394.

Hobson, K. A., McLellan, B. N. and Woods, J. G. (2000). Using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope to infer trophic relationships among black and grizzly bears in the upper Columbia River basin British Columbia. *Can. J. Zool.* **78**, 1332-1339.

Howell, D. J. (1974). Bats and pollen: physiological aspects of the syndrome of chiropterophily. *Comp. Biochem. Physiol. A* **48**, 263-276.

Kleiber, M. (1961). *The Fire of Life*. New York: Wiley.

Minagawa, M. and Wada, W. (1984). Stepwise enrichment of $\delta^{15}N$ along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. *Geochim. Cosmochim. Acta* **48**, 1135-1140.

Mizutani, H., Fukuda, M. and Kabaya, Y. (1992). ^{13}C and ^{15}N enrichment factors of feathers of 11 species of adult birds. *Ecology* **73**, 1391-1395.

Oelbermann, K. and Scheu, S. (2002). Stable isotope enrichment ($\delta^{15}N$ and $\delta^{13}C$) in a generalist predator (*Pardosa lugubris*, Araneae: Lycosidae): effects of prey quality. *Oecologia* **130**, 337-344.

Owens, N. J. P. (1987). Natural variations in ^{15}N in the natural environment. *Adv. Mar. Biol.* **24**, 389-451.

Peterson, B. J. and Fry, B. (1987). Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* **18**, 293-320.

Tieszen, L. L., Boutton, T. W., Tesdahl, K. G. and Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. *Oecologia* **57**, 32-37.

Vanderklift, M. A. and Ponsard, S. (2003). Sources of variationin consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. *Oecologia* **136**, 169-182.

Voigt, C. C. (2003). The energetics of reproduction in the nectar-feeding bat *Glossophaga soricina. J. Comp. Physiol. B* **173**, 79-85.

Voigt, C. C., von Helversen, O., Michener, R. and Kunz, T. H. (2003a). Validation of a non-invasive blood-sampling technique for doubly-labelled water experiments. *J. Exp. Zool.* **296A**, 87-97.

Voigt, C. C., Matt, F., Michener, R. and Kunz, T. H. (2003b). Low rates of carbon isotope turnover in tissues of two nectar-feeding bats. *J. Exp. Biol.* **206**, 1419-1427.

Webb, S. C., Hedges, R. E. M. and Simpson, S. J. (1998). Diet quality influences the $\delta^{13}C$ and $\delta^{15}N$ of locusts and their biochemical components. *J. Exp. Biol.* **201**, 2903-2911.