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Effect of diet quality on carbon and nitrogen turnover and isotopic discrimination in blood of a New World nectarivorous bat

L. Leticia Mirón M.[1], L. Gerardo Herrera M.[1,*], Nicte Ramírez P.[1] and Keith A. Hobson[2]

[1]*Departamento de Zoología, Instituto de Biología, UNAM, Apartado Postal 70-153, 04510, México, DF, México and* [2]*University of Saskatchewan, Department of Biology, Saskatoon, Saskatchewan S7N 0W0, Canada*

*Author for correspondence at present address: Estación de Biología Chamela, Instituto de Biología, UNAM, Apartado Postal 21, San Patricio, Jalisco, 48980, México (e-mail: gherrera@ibiologia.unam.mx)

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Summary

Diet composition of carbon and nitrogen (C:N) could affect diet–tissue isotopic discrimination and elemental turnover rate in consumers but studies that test the nature of these changes are scarce. We compared carbon and nitrogen isotopic discrimination and turnover rates in individuals of Pallas' long-tongued bats *Glossophaga soricina* fed diets with protein soya isolate or amaranth grains as their main source of protein. Diets were of similar protein biological value but the soya diet had higher nitrogen content (2.2%N) and lower C:N ratio (39.6) than the amaranth diet (1.3%N, C:N=40.5). Most bats on the soya diet gained body mass whereas most bats on the amaranth diet lost body mass. Half-lives of carbon (24.3±3.8 days) and nitrogen (25.6±4.4 days) in bats switched to the soya diet were very similar. In contrast, in the bats switched to the amaranth diet, carbon half-life (39.7±3.4 days) was longer than that of nitrogen (25.0±6.0 days). The enrichment in ^{15}N between diet and blood was higher when bats were fed the amaranth diet (4.4±0.2‰) than when they were fed the soya diet (3.3±0.2‰). Similarly, bats on the amaranth diet had higher ^{13}C enrichment (2.0±0.2‰) than bats on the soya diet (0.1±0.1‰). Our results support recent hypotheses of the effect of nutrition on diet–tissue isotopic discrimination and turnover rate, and further shows that blood stable isotope analysis is an adequate approach to track seasonal dietary shifts in wild bats.

Key words: blood, carbon-13, fractionation, nectarivorous bat, *Glossophaga soricina*, nitrogen-15, stable isotope.

Introduction

During the last decades, the measurement of naturally occurring isotopes of carbon and nitrogen in tissues of consumers has become a powerful tool for animal ecologists interested in dietary reconstructions (Ben-David et al., 1997a,b; Hobson et al., 2000). This approach has been used to track dietary shifts through time, but it relies on a number of assumptions that have not been experimentally tested in most of the species involved in field studies (Gannes et al., 1997). For example, dietary reconstructions assume that animals are in steady-state equilibrium with their diet. When animals in the wild switch between isotopically distinct diets, their tissues are not in equilibrium with the new diet and it becomes critical to know the time required for the tissue to reflect its new composition. We examined this issue by estimating the tissue-specific turnover rate of an element using the isotope as a tracer; this is particularly important when the study is aimed at determining the timing of dietary shifts. Field studies that use stable isotopes of carbon and nitrogen simultaneously usually assume close coupling in the turnover rate of these elements and, consequently, assume that they provide similar time frames for dietary reconstruction. Studies with wild animals also assume that the stable isotope composition of the consumer differs from the composition of its diet in a predictable way and, with some exceptions (e.g. Haramis et al., 2001), they frequently use discrimination factors derived for other taxa. Some of the experimental studies that test these assumptions show that diet composition could affect diet–tissue isotopic discrimination and turnover rate (Haramis et al., 2001; Pearson et al., 2003). The effect of diet on isotope discrimination has been documented mostly for nitrogen and it is described by two contrasting hypothesis: (1) nitrogen isotopic discrimination will increase as % nitrogen (N) increases and the carbon to nitrogen (C:N) ratio decreases in diets ('quantity hypothesis'; Pearson et al., 2003), and (2) nitrogen discrimination will decrease as dietary protein quality increases ('quality hypothesis'; Roth and Hobson, 2000). A recent meta-analysis supports the quality hypothesis, but rejects the quantity hypothesis (Robbins et al., 2005).

The effect of diet on elemental turnover rates has been explored only in a few studies in which turnover rates were estimated in animals fed different diets (Haramis et al., 2001; Pearson et al., 2003). The assumption that the turnover rates of carbon and nitrogen are closely coupled has found support in

some studies (Haramis et al., 2001; Bearhop et al., 2002, Evans Ogden et al., 2004; MacAvoy et al., 2005) whereas it has been rejected in others (Hobson and Bairlein, 2003; Haramis et al., 2001; Carleton and Martínez del Rio, 2005).

A good example of the effect of diet composition on isotope discrimination and turnover rate was recently reported in two species of nectarivorous bats, *Glossophaga soricina* and *Leptonycteris curasoae* (Voigt et al., 2003; Voigt and Matt, 2004). These bats were switched to an extremely low nitrogen diet (0.1%N). The most interesting finding was that carbon and nitrogen turnover rate estimates in whole blood were extremely long. Carbon half-life was 113–120 days whereas nitrogen half life was 274–514 days. These values are much higher than any other estimate in blood of birds and other small mammals (Hobson and Clark, 1992, 1993; Haramis et al., 2001; Bearhop et al., 2002; Hobson and Bairlein, 2003; Pearson et al., 2003; Evans Ogden et al., 2004; Carleton and Martínez del Rio, 2005; MacAvoy et al., 2005), and are comparable only to the values found for carbon in whole blood of catfish *Ictalurus punctatus* (180 days; MacAvoy et al., 2001) and carbon and nitrogen in long-nosed bandicoots *Perameles nasuta* (~90 days; Klaassen et al., 2004). Nectarivorous bats have high mass-specific metabolic rates (van Helversen and Reyer, 1984; Winter and van Helversen, 2001) whereas bandicoots have low metabolic rates (Klaassen et al., 2004) and catfish metabolism varies with ambient temperature (MacAvoy et al., 2001). Slow carbon turnover rate in bat blood was explained as a result of presumably long-lived erythrocytes (Voigt et al., 2003). In the case of nitrogen, slow turnover rates were explained as the result of the effect of the mixing of internal and external sources of nitrogen, additional fractionation of nitrogen isotopes and individual metabolic rates (Voigt and Matt, 2004).

Carbon and nitrogen stable isotope analysis has been relatively recently incorporated into the battery of tools used for studying feeding ecology of bats (DesMarais et al., 1980; Herrera et al., 1993, 1998, 2001a,b, 2002; Fleming et al., 1993; Fleming, 1995; Nassar et al., 2003). Some of these studies examined whole blood (Herrera et al., 2001a,b, 2002) and assumed that half-life of carbon in bat blood was similar to the value found in avian blood for individuals of similar body mass (e.g. ~12 days; Hobson and Clark, 1992), that carbon and nitrogen had similar turnover rates, and that diet–tissue discrimination values were similar to the average values found in other taxa (e.g. 3.4‰ for nitrogen and 1‰ for carbon). The unexpected findings of Voigt and his colleagues, however, cast serious doubts on the appropriate use of the isotopic approach in previous studies with wild bat populations.

Nectarivorous bats were fed a diet of corn syrup, cane sugar, agave syrup and *Opuntia* fruits in previous discrimination and turnover experiments (Voigt et al., 2003; Voigt and Matt, 2004). These diets were high in carbohydrates but extremely low in nitrogen (fruits were the main source of nitrogen and contained 0.7% nitrogen). In addition to its low nitrogen content, such diets most likely were deficient in some essential amino acids. Probably as a consequence of this low quality diet, bats lost an average of 8% of their body mass during the experiments, suggesting that bats had to mobilize body reserves (Voigt et al., 2003; Voigt and Matt, 2004). Although New World nectarivorous bats are well known to feed on flower nectars that mainly consist of sugars and small amounts of amino acids (Baker and Baker, 1982; von Helversen, 1993; Winter and von Helversen, 2001), pollen and insects are also part of the diet of *G. soricina*, *L. curasoae* and other species of nectarivorous bats (Carvalho, 1961; Alvarez and González, 1969; Howell, 1974; Heithaus et al., 1975; Lemke, 1984; Herrera et al., 2001b). Insects and the pollen of some chiropterophilous plants contain protein in high amounts and are an adequate source of amino acids (Howell, 1974; DeFoliart, 1992). A sugar-rich but protein-poor diet, as used by Voigt and his colleagues, might not be a good approximation to the diet of nectarivorous bats in the wild. Consequently, it is necessary to probe the assumptions of previous bat field isotopic studies with experimental diets that more closely resemble the quality of their natural diets.

In this study, we determined the turnover rates and diet–tissue discrimination factors of carbon and nitrogen in whole blood of the nectarivorous Pallas' long-tongued bat (*G. soricina* Pallas) using two diet-switching experiments. It is difficult to provide nectarivorous bats with diets that satisfy their nutritional requirements using the items that they feed on in the wild. For example, a single individual might include pollen from several species of plants and several species of insects during one daily foraging event (Heithaus et al., 1975) and these items are not easily accessible in laboratory facilities. We switched two groups of bats to synthetic diets with protein soya isolate or amaranth grains as the main source of protein. These items are not eaten by nectarivorous bats in the wild but are good sources of protein and are easily accessed under experimental conditions. Protein in soya isolate (76%; Carias et al., 1995) and amaranth grains (75–78%; Yañez et al., 1994) have similar biological values (the percentage of absorbed protein that is retained; Robbins, 1993) to the protein found in pollen and insects (~70%; Smith and Green, 1987; DeFoliart, 1992; Van Tets and Hulbert 1999). Both diets had higher nitrogen content (soya diet: 2.2%N, C:N=18.8; amaranth diet: 1.3%N, C:N=30.1) than the diet offered by Voigt and his colleagues and most likely offered a more balanced source of amino acids. We chose experimental diets after several trials that showed that bats remained healthy in the long term.

The purpose of the study was twofold. First, the study was aimed at obtaining turnover rates and discrimination factors for nectarivorous bats on diets with an adequate source of nutrients and that more closely represented the conditions that bats face in the wild. We also tested the relationship between body mass changes and carbon and nitrogen turnover rates. We predicted that turnover rates would be slower in bats that lost more mass. The second aim of the study was to test the effect that diets with similar protein biological value but different nitrogen concentration had on carbon and nitrogen turnover rates, metabolic coupling of carbon and nitrogen and diet–tissue discrimination factors. According to the quality hypothesis, there should no be differences in diet–tissue discrimination in

both experimental diets. However, if the predictions of the quantity hypothesis were right, discrimination for nitrogen (and probably carbon too) should be higher in the soya diet. We expected close coupling between nitrogen and carbon metabolism with bats on the soya diet because bats on this diet are able to keep a positive nitrogen balance (L.M.M. and L.G.H., unpublished observations) thus reducing the need to using endogenous sources of this nutrient. Longer nitrogen turnover rates were expected on the amaranth diet than the soya diet if its lower nitrogen content forced bats to use endogenous nitrogen sources. Consequently, we considered that uncoupling of carbon and nitrogen metabolism was more likely to occur in bats on the amaranth diet.

Materials and methods

The sample

Juvenile bats (*Glossophaga soricina* Pallas) were captured in Mexico in caves in Teloloapan (99°52′W, 18°22′N), Morelos in March 2001 (Group 1, *N*=8, 6 females, 2 males) and in Tenampulco (97°24′W, 20°08′N), Puebla in September 2001 (Group 2, *N*=5, all males). Bats from each group and sex were placed in separate 0.80 m×0.80 m×0.80 m cages in a room at an ambient temperature of 25-27°C and 30% humidity. Illumination was kept at an artificial light:dark cycle of 12:12 h. Animals were fed a diet of cereal, table sugar, powdered milk and banana ('milk diet'; Table 1) for 365 days and 165 days for groups 1 and 2, respectively. Water was provided *ad libitum* during this period.

Experimental protocol

The first group was fed the milk diet, and the second group was fed a mixture of soy protein extract, cereal, table sugar and banana diluted in water ('soya diet'; Tables 1 and 2) for an additional 18 months. After this period, bats on the milk diet were switched to the soya diet, whereas bats on the soya diet were switched to a mixture of amaranth, sucrose, cereal and banana diluted in water ('amaranth diet'; Tables 1 and 2). Bats were kept on the experimental diets for 105 days and their body mass was measured every week with an electronic balance (Ohaus®) to the nearest mg. Each component of the diet was mixed at the beginning of the study to offer isotopically uniform diets. In the case of banana, we mixed the estimated mass needed for the whole experiment and separated portions for each day of the study. Banana portions were frozen and used as needed.

Table 1. *Composition of experimental diets*

Diet	Type	Amount
Milk	Powdered cow's milk	11.5 g
	Water	475.4 ml
Soy	Soy protein extract	3.7 g
	Water	483 ml
Amaranth	Amaranth grains	16.9 g
	Water	470 ml

Each diet was made with 15.3 g of cereal, 90.1 g of banana, and 7.6 g of sucrose in addition to the components listed above.

We were most concerned with not introducing problems of nutritional quality during the diet switch and so were constrained in terms of what dietary options we could consider. Previous studies have used relatively small isotopic differences (Hobson and Bairlein, 2003; Pearson et al., 2003) and our diets differed isotopically within this range (Table 3). Milk and soya diets differed by 1.3‰ for $\delta^{15}N$ and by 2.3‰ for $\delta^{13}C$, whereas soya and amaranth diets differed by 1.5‰ for $\delta^{15}N$ and by 4‰ for $\delta^{13}C$ measurements. Soya and amaranth diets had similar biological value but differed in nitrogen and carbon content (Table 3). C:N ratios were the highest in the amaranth diet followed by milk and soya diets in that order.

Blood (~50–80 μl) was extracted from the antebrachial vein of the bats at 0, 2, 4, 8, 21, 35, 48, 62, 77, 90 and 105 days. We stopped blood flow after collecting the sample by pressing a finger on the punctured point. Arms were used alternately for sampling. Individual bats were bled either at 2 or 4 days after the beginning the experiment to prevent stressing the bats with excessive handling. Afterwards, bats were bled weekly or bi-weekly. Blood was placed in plastic vials with 1 ml of 70% ethanol, dried at 40°C to a constant weight and kept refrigerated until analysis. Samples of the food offered were collected throughout the experiment, dried and stored in a refrigerator (–10°C).

Stable isotope analysis

Stable isotope analyses were conducted at the Soil Science Laboratory at the University of Saskatchewan, Canada. Dried samples were powdered in a small mortar and pestle. Samples of about 1 mg were then weighed into tin cups and combusted in a Robo-Prep™ elemental analyzer (Manchester, UK) at 1200°C. Resultant gases were separated and analyzed in a Europa 20:20™ continuous-flow isotope ratio-mass spectrometer (CFIRMS; Manchester, UK) for stable-carbon and nitrogen isotope ratios on the same sample. CFIRMS involved the automated sequential measurement of samples

Table 2. *Experimental protocol*

Group 1				Group 2			
Diet 1	Days	Diet 2	Days	Diet 1	Days	Diet 2	Days
Milk	540	Soya	105	Soya	540	Amaranth	105

Each group of bats was switched to an experimental diet (Diet 2) after being fed a pre-experimental diet (Diet 1).

Table 3. *Carbon and nitrogen content, isotopic composition and biological value of protein of experimental diets*

Diet	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	%N	%C	C:N	%BV*	*N*
Milk	3.8±0.8	–20.6±0.2	1.8±0.5	42.1±2.4	24.1±4.5	96[1]	7
Soya	2.5±0.3	–22.9±0.6	2.2±0.5	39.6±2.1	18.8±4.1	75[2]	8
Amaranth	4.0±0.4	–18.9±0.5	1.3±0.1	40.5±1.1	30.1±2.2	77[3]	9

Values are mean ± s.d.
*BV (biological value) is the percentage of absorbed protein that is retained (Robbins, 1993).
[1]MacDonald et al. (1973); [2]Carias et al. (1995); [3]Yañez et al. (1994).

Table 4. *Percentage body mass change and turnover rate and half-life of carbon and nitrogen in whole blood of bats fed a soya-based diet*

		C		N	
Sex	ΔM_b	*b*	t_{50} (days)	*b*	t_{50} (days)
M	–7	0.028	24.5	0.022	30.5
F	11	0.023	30.1	0.022	30.2
F	25	0.032	21.3	0.027	24.8
F	14	0.036	19.2	0.035	19.7
M	–5	0.029	23.9	0.028	24.7
F	–13	0.024	27.9	0.022	30.4
F	4	0.033	20.8	0.033	20.9
F	0	0.026	26.6	0.030	23.1
Mean ± s.d.	3.6±12	0.029±0.004	24.2±3.7	0.027±0.004	25.5±4.3

ΔM_b, body mass change; *b*, turnover rate ; t_{50}, half-life.
Bats were fed this diet for 105 days after being on a milk-based diet for ~30 months. M, male; F, female.

(unknowns) together with reference material. We used two laboratory standards (egg albumen) for every five unknowns in sequence. Stable-isotope ratios were expressed in δ-notation as parts per thousand (‰) deviations from the international standards PDB (carbon) and air (nitrogen) according to the equation:

$$\delta X = [(R_{sample} – R_{standard}) – 1] \times 1000 , \quad (1)$$

where X was ^{13}C or ^{15}N and *R* was the corresponding ratio ^{13}C:^{12}C or ^{15}N:^{14}N. Based on several hundred replicates of laboratory standards, we estimated laboratory measurement error to be ±0.3‰ and ±0.1‰ for stable nitrogen and carbon-isotope values, respectively.

Turnover rates and trophic discrimination

We obtained suitable isotopic dietary shifts to allow us to model turnover rates for both $\delta^{15}N$ and $\delta^{13}C$ values. We fitted the isotopic data to equations of the form:

$$Y(t) = Y_a + a[\exp(–bt)] \quad (2)$$

using Sigmaplot (Version 5). Here *Y(t)* is the isotopic value of blood at time *t*, Y_a represents the asymptotic tissue isotope

Table 5. *Percentage body mass change, turnover rate and half-life of carbon and nitrogen in whole blood of bats fed an amaranth-based diet*

		C		N	
	ΔM_b	*b*	t_{50} (days)	*b*	t_{50} (days)
	–2	0.017	40.1	0.027	25.3
	–10	0.019	35	0.023	23.1
	–5	0.015	43.5	0.027	25.7
	–11	0.018	37.6	0.040	17
	5	0.016	42	0.020	33.6
Mean±s.d.	–4.9±6.7	0.029±0.004	39.6±6.3	0.027±0.004	24.9±5.9

ΔM_b, body mass change; *b,* turnover rate; t_{50}, half-life.
Bats were fed this diet for 105 days after being on a soya-based diet for ~18 months.
All bats were males.

Table 6. *Spearman rank correlation coefficients for the relationship between carbon and nitrogen turnover rates and body mass change in Glossophaga soricina*

Diet	Element	r_s	*P*
Amaranth	Carbon	–0.60	0.28
	Nitrogen	–0.91	0.03
Soya	Carbon	0.47	0.23
	Nitrogen	0.52	0.18

Level of significance was Bonferroni-adjusted to 0.025.

value, *a* is the absolute difference between the initial and asymptotic condition, *b* is the turnover rate of carbon or nitrogen in blood, and *t* is time since diet switching. To calculate the half-life of each element, exponential curves were fitted for each individual. Half-life (t_{50}) was defined as –ln(0.5)/*b* and individual bat values were averaged for each element in each experiment. Trophic discrimination for carbon- and nitrogen-stable isotopes was estimated for each individual on each diet as the difference between average isotopic values of the diet and the derived asymptotic isotopic values (Y_a).

Statistical analysis

We used non-parametric tests to evaluate the relationship between body mass changes and turnover rate, and to compare turnover rates and enrichment factors between diets. The level of significance for these tests was adjusted to 0.025 using Bonferroni correction because two data sets were used for each individual.

Results

Body condition

On average, bats on the soya diet gained body mass over the course of the experiments although three bats lost mass (Table 4). In contrast, average mass at the end of the experiment decreased in bats on the amaranth diet (Table 5). We did not observed evidence of significant loss of hair in individuals on any diet.

Turnover rates and trophic discrimination

Nitrogen turnover rates did not differ between diets (Mann–Whitney *U*=19, *P*=0.88) but carbon turnover rate was higher with the amaranth diet (Mann–Whitney *U*=0, *P*=0.003). We estimated a half-life (mean ± s.d.) of 25.5±4.3 days for δ^{15}N values and of 24.2±3.7days for δ^{13}C values for the bats switched to the soya diet (Table 4). In the case of bats switched to the amaranth diet (Table 5), half life of δ^{15}N and δ^{13}C values were 24.9±5.9 and 39.6±6.3 days, respectively. Carbon and nitrogen turnover rates were not affected by body mass change in any of the diets (Table 6). For purely illustrative purposes, we depicted elemental turnover patterns for nitrogen and carbon by fitting a single decay curve to the means of the combined data for all individuals for each diet switch (Fig. 1). The enrichment in ^{15}N between diet and blood was higher

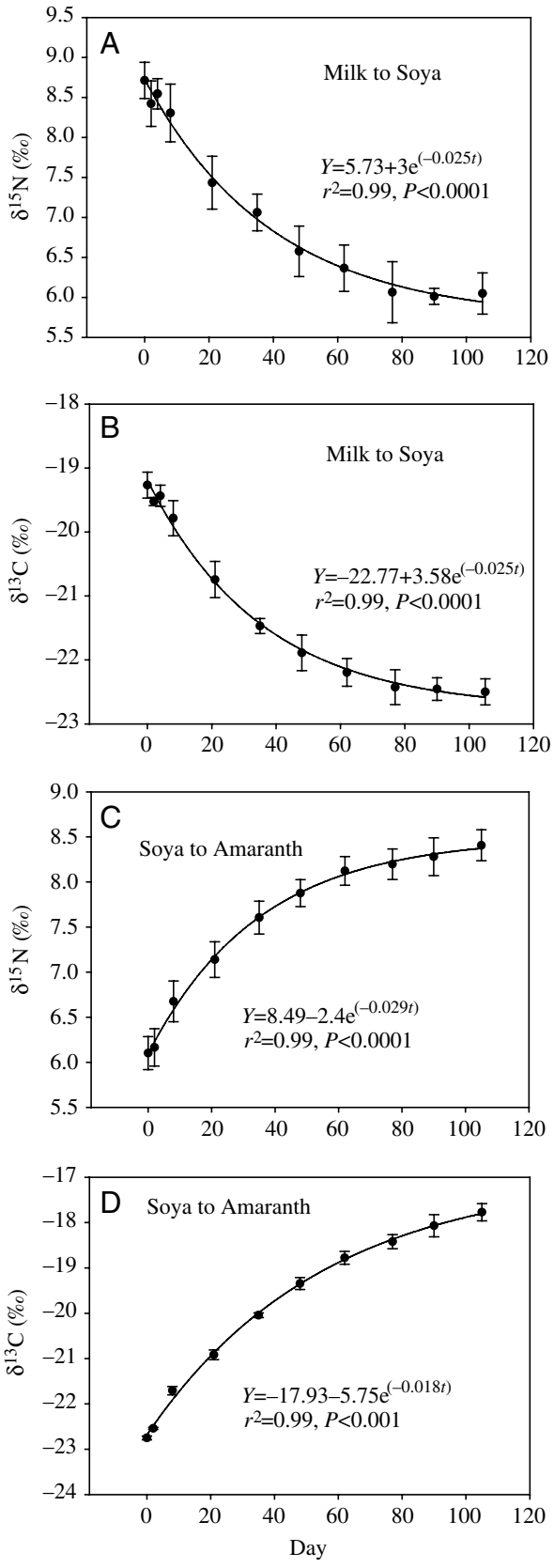

Fig. 1. Isotopic results (mean ± s.d. for δ^{15}N and δ^{13}C in ‰) of the diet switch from the milk-based to the soya-based diet (A,B), and from the soya-based to the amaranth-based diet (C,D).

Table 7. *Carbon and nitrogen half-lives reported in the literature for blood of one rodent and several species of birds*

Species	Ct_{50} (days)	Nt_{50} (days)	Body mass (g)	Source
House mouse	17	19	18	MacAvoy et al., 2005
Great skua	14	15	1218	Bearhop et al., 2002
Dunlin	11	10	56	Evans-Ogden et al., 2004
Garden warbler	11	5	20	Hobson and Bairlein, 2003
Canvasback	16–26	17–26	1248	Haramis et al., 2001
House sparrow	15	23	23	Carleton and Martínez del Rio, 2005
American crow	29		428	Hobson and Clark, 1993
Japanese quail	11		190	Hobson and Clark, 1992

Ct_{50}, carbon half-life; Nt_{50}, nitrogen half-life.

when bats were fed the amaranth diet (4.4±0.2‰) than when they were fed the soya diet (3.3±0.2‰, U=0, P=0.003). Similarly, bats on the amaranth diet had a higher ^{13}C enrichment (2±0.2‰) than bats on the soya diet (0.1±0.1‰, U=0, P=0.003).

Discussion

Turnover rate and diet quality

Bats switched to the soya diet had average half-lives of 25 and 24 days for nitrogen and carbon, respectively, whereas average half-lives in the bats switched to the amaranth diet were 25 and 39 days, respectively. Because most bats on the soya diet were females and all bats on the amaranth diet were males, sex could have influenced our results. However, this is unlikely given that the two males on the soya diet had half-life values similar to the females. Carbon turnover rates in our study were not very different than values in blood of one rodent and several species of birds (Table 7). In contrast, carbon and nitrogen half-life values in this study were shorter than the estimates obtained in previous studies with two species of nectarivorous bats, in which true turnover rates were probably obscured because of the mixing of internal and external sources of nitrogen (and carbon), and effects of additional fractionation of nitrogen (and carbon) isotopes because of diet quality (Voigt et al., 2003; Voigt and Matt, 2004). Unlike the work of Voigt and his colleagues (Voigt et al., 2003; Voigt and Matt, 2004), we collected blood from the same individual on the second or the fourth day after beginning the experiment. It remains to be tested whether this additional collection of blood increased the rate of synthesis of blood cells. However, blood volumes were the minimum for analysis and other studies with small birds have used the same blood sampling protocol (Hobson and Bairlein, 2003; Pearson et al., 2003; Carleton and Martínez del Rio, 2005).

Faster turnover rates in *G. soricina* in our study compared to previous bat studies (Voigt et al., 2003; Voigt and Matt, 2004) were probably explained by the low-quality diet used in those previous studies. Bats in the wild complement their nectar diet with insects and pollen and these items are rich sources of protein (Howell, 1974; Smith and Green, 1987; DeFoliart, 1992; van Tets and Hulbert, 1999). Thus, unlike bats fed a nitrogen-poor diet (0.1%N) in the experiment by Voigt and his colleagues, nectarivorous bats have natural diets that include protein-rich items. In our study, bats were fed diets with higher nitrogen content (1.3–2.2%N) and with protein sources of biological values (75–78%) similar to the protein of insects and pollen (~70%).

Half-life estimates of carbon in our study provide a time window of dietary integration of 48–78 days (e.g. two half-lives; Hobson and Clark, 1993). In the case of nitrogen, the time window amounts to ~50 days. These estimates are about twice the values assumed in previous studies that analyzed whole blood isotope composition of wild bats at different times of the year (e.g. 23 days; Herrera et al., 2001a,b, 2002) but they suggest that the blood isotope analysis is an adequate method to track seasonal dietary changes in these animals. Because we conducted our experiments with groups of bats restrained to a 0.5 m^3 cage, it is probable that our results underestimated turnover rates in wild animals with potentially higher field metabolic rates. However, house sparrows *Passer domesticus* have similar carbon and nitrogen turnover rates in red blood cells even when their resting metabolic rate differs by a factor of 2 (Carleton and Martínez del Rio, 2005) and increased metabolism does not necessarily equate with increased elemental turnover through blood cell replacement.

Turnover rate and body condition

In the present study, the amaranth diet had lower nitrogen content than the soya diet which probably explains body mass losses of bats on this diet. However, the poorer body condition of bats at the end of the experiment on the amaranth diet compared to bats on the soya diet did not affect turnover rate of nitrogen because Nt_{50} were not different. In contrast, turnover rate of carbon was slower in bats on the amaranth diet than on the soya diet (e.g. Ct_{50} was 62% higher in the amaranth diet). Similar to previous findings with *G. soricina* and *L. curasoae* (Voigt et al., 2003; Voigt and Matt, 2004), carbon and nitrogen turnover rates did not increase with body mass losses in any of the diets, which indicates that higher Ct_{50} in bats on the amaranth diet are not simply a result of an increasing use of endogenous sources. However, one must be

cautious in the case of the relationship between nitrogen turnover rate and body mass change on the amaranth diet because of the conservative nature of the Bonferroni correction. A less conservative method (Rice, 1989) could lead to a different conclusion for this.

Coupling between carbon and nitrogen dynamics

When bats were fed the soya diet, there was a close coupling between carbon and nitrogen turnover rates similar to that reported in other vertebrates (Haramis et al., 2001; Bearhop et al., 2002; Evans Ogden et al., 2004; MacAvoy et al., 2005). In contrast to our initial predictions, carbon in the amaranth diet had slower turnover rates than nitrogen, indicating the effect of decoupling of their metabolic pathways during blood cell formation. Decoupling of carbon and nitrogen turnover rates was reported in whole blood of garden warblers *Sylvia borin* switched from mealworms to a fruit diet (Hobson and Bairlein, 2003), canvasback *Aythia valiniseria* fed a high carbohydrate diet (Haramis et al., 2001), and house sparrows fed a corn diet (Carleton and Martínez del Rio, 2005). Unlike bats on the amaranth diet, Nt_{50} was higher than Ct_{50} in these studies. However, in liver of house mouse *Mus musculus*, Ct_{50} was much higher than Nt_{50}, suggesting that carbon turnover reflected total tissue (e.g. carbohydrates, lipids, proteins and nucleic acids) turnover whereas nitrogen turnover reflected primarily protein turnover (MacAvoy et al., 2005). Accordingly, Ct_{50} values in the amaranth diet were probably a result of carbon turnover of other molecules in addition to protein. Our results confirmed the assumption in previous studies with wild bats that carbon and nitrogen have similar turnover rates (Herrera et al., 2001a,b) but this may not be true when diets are deficient in some nutrient (e.g. our amaranth diet) leading to slower carbon turnover rates.

Isotopic discrimination

Diet–tissue nitrogen enrichment (3.3±0.2‰) for the soya diet (2.2±0.5%N) was similar to the average value reported in the literature for mammals (3.3±1.3‰; Robbins et al., 2005) under different diets (5.3±3.6%N) and to the value found in blood of *G. soricina* (3.2±1.3‰) fed a nectar–pollen diet (1.3%N; Voigt and Matt, 2004). Our diet–tissue nitrogen enrichment value was similar to that used in previous isotopic studies with wild bats (Herrera et al., 2001a, 2003). However, when bats were fed the amaranth diet (1.3±0.1%N), nitrogen enrichment was significantly higher (4.4±0.2‰). Pearson et al. (2003) hypothesized that nitrogen isotopic discrimination increased as %N increased and C:N ratio decreased in diets (the quantity hypothesis). In contrast, Roth and Hobson (2000) predicted that nitrogen isotopic discrimination should decrease as dietary protein quality increases (the quality hypothesis). Because we used diets with protein of similar biological value, we did not expect nitrogen isotopic discrimination to differ between them. On the contrary, we found higher ^{15}N enrichment in bats fed the diet with lower %N and higher C:N ratio, in contradiction of the predictions of the quantity hypothesis. Similarly, we found that carbon isotope discrimination was higher in the amaranth (2.0±0.2‰) than in the soya (0.1±0.1‰) diet. Diet–tissue carbon isotope discrimination associated with the amaranth diet was similar to the value reported previously for *G. soricina* on a nectar–pollen diet (2.3‰; Voigt et al., 2003). As suggested by the pattern found in carbon and nitrogen turnover in bats on the amaranth diet, a significant portion of carbon incorporated into blood probably originated from non-protein carbon, thus leading to higher carbon discrimination factors than the soya diet, in which most carbon was probably derived from protein metabolism.

In summary, our results showed that diet–tissue fractionation and turnover rates were influenced by diet quality but not to the extent previously reported for two species of nectarivorous bats (Voigt et al., 2003; Voigt and Matt, 2004). The assumptions of previous studies with wild bats were supported at least in one of the diets offered in our study, which indicates that blood stable isotope analysis is an adequate approach to track seasonal dietary shifts.

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