Nectar-feeding bats fuel their high metabolism directly with exogenous carbohydrates

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Summary

1. Mammals usually derive energy from metabolizing fat and glycogen stores combined with exogenous food. Nectarivorous bats mostly consume a diet low in both fat and proteins but rich in simple carbohydrates. Metabolizing exogenous carbohydrates directly to fuel their high mass-specific metabolic rate would save the energetic costs of lipogenesis and gluconeogenesis for nectarivorous bats. Therefore, we expected nectarivorous bats to switch to exogenous carbohydrates rapidly when available and use them predominantly instead of fat or glycogen.

2. We first investigated the rate of fractional incorporation of dietary sugars into the pool of metabolized substrates in Glossophaga soricina by measuring the change in $\delta^{13}$C enrichment of exhaled CO$_2$ ($\delta^{13}$C$_{\text{breath}}$) when animals were fed glucose, fructose or sucrose that was isotopically distinct from their normal diet. Second, we performed a diet-switch experiment to estimate the turnover rate of fat tissue.

3. When fed with sugars, the $\delta^{13}$C$_{\text{breath}}$ converged quickly on the isotope signature of the ingested sugars, indicating an almost exclusive use of dietary carbohydrates. The time for a 50% carbon isotope exchange in exhaled CO$_2$ equalled 9, 13 and 14 min for fructose, glucose and sucrose, respectively. Nectarivorous bats fuelled 82% of their metabolism with exogenous carbohydrate when fed with fructose, 95% when fed with glucose and 77% when fed with sucrose. Bats depleted 50% of their fat stores each day.

4. Although nectarivorous bats consumed most of their body fat each day, this was still barely enough to sustain their diurnal metabolism. The fractional incorporation rates of dietary sugars into the pool of metabolized substrates in G. soricina are the fastest rates ever found in a mammal.

Key-words: breath, exhaled CO$_2$, Glossophaga soricina, stable carbon isotopes

Introduction

Neotropical nectarivorous bats (Glossophaginae; Phyllostomidae) are among the smallest living mammals (< 10 g) and are specialized on the exploitation of flowers (Von Helversen & Winter 2003). Similar to hummingbirds, glossophagine bats consume floral nectar using an energetically costly hovering flight mode (Von Helversen 1986; Voigt & Winter 1999). Glossophagine bats have higher mass-specific metabolic rates than similar-sized terrestrial mammals (Nagy, et al. 1999; Speakman 2000) or similar sized bats with other feeding habits (McNab 1969, 1989; Von Helversen & Reyer 1984; Voigt, Kelm & Visser 2006). Floral nectars of chiropterophilous plants are dilute (Dobat & Peikert-Holle 1985), and consist mostly of sucrose and the monosaccharides glucose and fructose (Baker, Baker & Hodges 1998). These simple sugars are rapidly digested and/or absorbed, hence appropriate for fuelling costly locomotion modes such as hovering flight (e.g. hummingbirds: Suarez et al. 1990; Suarez & Gass 2002).

Glossophagine bats consume large amounts of dilute nectar each night (Von Helversen & Winter 2003); a 10-g nectarivorous bat may ingest up to 150% of its body mass as nectar (Von Helversen & Reyer 1984; Voigt et al. 2005, 2006). In response to their sugary diet, glossophagine bats have large amounts of sucrase in their digestive tract (Hernandez & Martinez del Rio
those of terrestrial granivorous rodents (a few weeks in the digestive tracts (Hernandez & Martínez del Rio 1992). Consequently, although fat allows the bats to store energy, fat tissue has several disadvantages in these animals. Therefore, we expected nectarivorous bats to combust mostly carbohydrates from ingested sugar rather than using endogenous substrates such as glycogen or fat. We hypothesized that glossophagine bats would fuel their energetically expensive life directly with dietary carbohydrates, much in the same way as hummingbirds do. We measured the stable carbon isotope ratio in exhaled CO₂ (δ¹³C breath) of Glossophaga soricina to determine whether δ¹³C breath originates either from exogenous sugars, that is, sucrose, fructose and glucose, or from isotopically distinctive labelled endogenous substrates, that is, fat (Hatch, Pinshow & Speakman 2002a,b). We predicted that the δ¹³C breath of starved nectarivorous bats that had recently fed on sugar water would rapidly converge on the isotopic signature of exogenous sugars, if the bats switched their metabolic substrate use predominantly to the ingested sugars. Alternatively, if nectar-feeding bats use a mixture of endogenous and exogenous substrate, δ¹³C breath should stabilize at a level intermediate between the δ¹³C of the two isotopically distinct carbon sources. Furthermore, we predicted that G. soricina would incorporate dietary sugars into the pool of metabolized substrates at rates similar to those of hummingbirds (Carleton, Bakken & Martínez del Rio 2006; Welch et al. 2006), and we expected that the fractional incorporation rate of sucrose would be similar to that of the two monosaccharides (glucose and fructose), since sucrase is abundant in the bats’ digestive tracts (Hernandez & Martinez del Rio 1992). Finally, we predicted turnover rates of fat tissues in nectarivorous bats to fall closer to those of hummingbirds (1–2 days in the broad-tailed hummingbird Selasphorus platycercus; Carleton et al. 2006) than to those of terrestrial granivorous rodents (a few weeks in the Mongolian gerbil Meriones unguiculatus: Tieszen et al. 1983) owing to the high metabolic rate and small fat deposits of nectarivorous bats (McNab 1976; Von Helversen & Reyner 1984; Voigt et al. 2006).

Methods

We captured 56 individuals (27 males, 29 females) of G. soricina, Pallas (Glossophaginae, Phyllostomidae) from a captive breeding colony maintained in greenhouse facilities at the University of Erlangen-Nuremberg (Germany). Animals were assigned arbitrarily to two treatments, that is, they were either fed a diet based on C₄/CAM plant products (groups A and B) or a diet based on C₃ plant products (groups C, D and E) for 20 days prior to the actual measurements. Group A consisted of 8 bats, group B of 16 bats, group C of 16 bats, and groups D and E of 8 bats each. The sizes of flight rooms were either 2 × 2 × 3 m³ or 5 × 3 × 4 m³. The C₄/CAM plant based sugary diet of groups A and B was based on 18% mass/mass Agave syrup water to which we added supplemental food (Nido Superkid, Nestlé, South Africa) and the C₃ plant based sugary diet of groups C, D and E was based on 18% (m/m) honey water to which we added Altemil (Alete, Nestlé, Germany). The C₃/CAM plant based diet consisted of c. 62% fructose, 16% glucose and 22% sucrose. The C₃ plant based diet consisted of c. 32% fructose, 28% glucose and 40% sucrose. These values were calculated according to the ratio at which the base diet (either Agave syrup or honey) was mixed with the supplemental food (Nido Superkid and Altemil) and according to the manufacturers’ specifications. The dietary stable carbon isotope ratio of groups A and B was −11.6‰ and that of groups C, D and E −26.0‰ (Table 1). Animals from groups A and B are called C₄ bats and animals from groups C, D and E are called C₃ bats. Ambient temperature in the flight rooms was 27 °C, relative humidity 70% and the photoperiod was 12:12 h. Individuals of each group were marked by labelling the toes with white marker fluid (Tipp-Ex, Nodef, Germany) prior to the experiment. After the study all individuals were returned to the breeding colony. The average body mass of the bats was 9.9 ± 1.1 g (n = 56).

Table 1. Experimental treatment of bat groups, average volume of sugar water ingested (mL) per individual and experiment (± 1 standard deviation) and stable carbon isotope signature of sugars (‰)

<table>
<thead>
<tr>
<th>δ¹³C of maintenance diet (‰)</th>
<th>Sugar switched to during experiment</th>
<th>Group</th>
<th>Sugar water ingested (mL)</th>
<th>δ¹³C sugar (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−11.6</td>
<td>C₄ fructose</td>
<td>B</td>
<td>1.61 ± 0.42</td>
<td>−26.77</td>
</tr>
<tr>
<td></td>
<td>C₃ glucose</td>
<td>A</td>
<td>1.56 ± 0.32</td>
<td>−26.52</td>
</tr>
<tr>
<td></td>
<td>C₃ sucrose</td>
<td>B</td>
<td>1.55 ± 0.15</td>
<td>−25.65</td>
</tr>
<tr>
<td></td>
<td>C₄ fructose</td>
<td>C</td>
<td>1.55 ± 0.32</td>
<td>−10.65</td>
</tr>
<tr>
<td></td>
<td>C₃ glucose</td>
<td>D</td>
<td>1.61 ± 0.22</td>
<td>−10.56</td>
</tr>
<tr>
<td></td>
<td>C₄ sucrose</td>
<td>C</td>
<td>1.49 ± 0.25</td>
<td>−12.23</td>
</tr>
</tbody>
</table>
Fractional Incorporation of Dietary Sugars into the Pool of Metabolized Substrate

The fractional incorporation of dietary sugars into the pool of metabolized substrates was assessed using a diet-switching protocol on bats in groups A–D. During each experimental day we tested only a single type of sugar. We performed two experiments each with a specific sugar with bats of group A and B. The two experimental days were separated by 1 day. Details of the switching protocol for each sugar and carbon source are depicted in Table 1. Each experimental run lasted 60 min and consisted of six breath collection events. For breath sampling, bats were transferred singly into silk bags (1 × 7 cm²) that were put each into a larger plastic bag (10 × 10 cm²; volume 200 mL; Ziploc™). Ambient air was washed of CO₂ using NaOH and flushed through the silk bag via a plastic tube (diameter 3 mm) at a flow through rate of 700 mL min⁻¹. The outlet of the plastic bag consisted of a small slit of 4 cm (width 0·2 cm). Then we sealed the plastic bag for 1·5 min to let CO₂ accumulate in it. *Glossophaga soricina* have a resting metabolic rate of c. 1·14 mL O₂ min⁻¹ (Cruz-Neto & Abe 1997). Therefore, we expected CO₂ to accumulate to c. 0·5% during this time span. For breath collection we used evacuated vacutainers (Labco™, Buckinghamshire, UK). Approximately 10 mL of air were sucked from close to the position of the bat in the silk bag via a second plastic tube (diameter 1 mm, length 4 cm) when a needle hermetically fused to the tube’s end outside the bag penetrated the Teflon membrane of a vacutainer. After each breath collection the plastic bag was unsealed again and CO₂ free air was flushed through the bag. Breath collection was repeated after 5, 10, 20, 40 and 60 min following the first feeding event, since we expected an exchange of stable carbon isotopes in exhaled CO₂ during this time period. Bats were fed repeatedly after 20 and 40 min following the first feeding event to ensure that the bats’ breath was equilibrated isotopically to the new diet. The average amount of ingested sugar water per individual is listed in Table 1. On each experimental day, bats were fed with a single type of sugar water, either mixed with C₆ labelled fructose, glucose (both Sigma Aldrich, Munich, Germany) and sucrose (beet sucrose; Kristallzucker, Südzucker, Germany) or C₁₂ labelled fructose, glucose and sucrose (commercially available sugars based on corn). Sugar was mixed with water to a concentration of c. 30% (mass/mass; Atego refractometer, Atego Ltd, Tokyo, Japan). To measure baseline stable carbon isotope ratios of exhaled CO₂ in unfed animals, we collected breath samples of five unfed individuals from groups A and C at the same time intervals as the other experiments, and tested whether stable carbon isotope signatures of exhaled CO₂ changed during the course of the experiment using a repeated measures ANOVA. All bats were released into the flight rooms after experimental runs and afterwards to the breeding colony in the greenhouses.

Turnover of Fat Tissue

We changed the diet of group E for 4 days from C₃ plant products to C₄/CAM plant products (same diet as previously used for equilibration of groups A and B). On the first (day 0) and last day (day 3) of the diet switch experiment all bats were weighed (electronic balance, Mettler PM-100, Greifensee, Switzerland, accuracy 0·01 g). Between 17:00 and 19:00 h preceding day 0 of the experiment, and at the same time of day on the following 3 days, bats were caught with hand nets and put singly into silk bags. Breath collection followed the procedure described above except we collected only a single breath sample and released the animals immediately afterwards into their flight cage.

Isotope Analysis

Stable carbon isotope ratios of sugar samples were measured following the description in Voigt & Kelm (2006) at the Department of Geology and Mineralogy of the University of Erlangen-Nürnberg. Breath samples were measured in an ISOCHROM-μG isotope ratio mass spectrometer (Micromass, UK; Perkins & Speakman 2001). The breath samples were automatically flushed from the vacutainers in a stream of chemically pure helium, after which a gas chromatograph separated the CO₂ gas from the other gases before admitting it into the mass spectrometer in a continuous flow. Breath samples together with internal standards that had been previously characterized relative to an international ¹³C standard (IAEA-CO-1) were analyzed in duplicates. All ¹³C/¹²C were expressed relative to the international standard using the δ notation in parts per mil (%) and the following eqn 1:

\[ \delta^{13}C(\%) = \left( \frac{^{13}C}{^{12}C} \right)_{\text{sample}} \left[ \frac{^{13}C}{^{12}C} \right]_{\text{IAEA-CO-1}} - 1 \times 10^3 \]

with ¹³C/¹²C representing the isotope ratio in either the breath sample or the standard. Precision was better than ±0·01‰ (1σ). All samples were analyzed using a blind experimental protocol. Carbon dioxide concentration was too low in some samples of one individual of group B that was fed C₁₂ fructose and therefore we excluded this individual from further analysis.

Calculation of Equation Parameters

We expected that changes in isotopic composition follow a single-pool exponential model (e.g. Tieszen *et al.* 1983; Voigt *et al.* 2003). Hence, we calculated equations of the following type for each individual and experiment according to Carleton & Martínez del Rio (2005):

\[ \delta^{13}C_{\text{breath}}(t) = \delta^{13}C_{\text{breath}}(\infty) + \left[ \delta^{13}C_{\text{breath}}(0) - \delta^{13}C_{\text{breath}}(\infty) \right] e^{-kt} \]
In eqn 2, $\delta^{13}C_{\text{breath}}(t)$ is the stable carbon isotope ratio of exhaled CO$_2$ at time $t$, $\delta^{13}C_{\text{breath}}(\infty)$ the asymptotic stable carbon isotope ratio of exhaled CO$_2$ when animals are equilibrated to the stable carbon isotope signature of their diet, $\delta^{13}C_{\text{breath}}(0)$ the stable isotope ratio of exhaled CO$_2$ at time 0 of the experiment, and $k$ the fractional rate of isotope incorporation into exhaled CO$_2$. Estimation of $k$ was performed on an iterative basis using SigmaPlot (spss, Version 8.0). For each experiment, we averaged regression coefficients over individual values. To test for differences in mean $\delta^{13}C_{\text{breath}}(\infty)$ and the stable carbon isotope ratio of dietary sugars we performed one sample Student’s $t$-tests.

We calculated the time at which 50% of carbon isotopes are exchanged in the animals’ breath ($t_{50}$) according to the following equation: $t_{50} = -\ln(0.5)/k$, with ln representing the natural logarithm and 0.5 the exchange of 50% isotopes. All values are given as means ± 1 standard deviation and all statistical tests were performed two-tailed if not stated otherwise.

In the fat tissue turnover experiment, we estimated the asymptote value of $\delta^{13}C_{\text{breath}}(\infty)$ of eqn 2 in the following way, since the asymptotic increase of $\delta^{13}C_{\text{breath}}$ did not reach a plateau. We averaged the initial values of unfed bats equilibrated to the C$_4$ plant based diet (groups A and B; $n = 24$) and the C$_4$/CAM plant based diet (groups C and D; $n = 24$) and calculated the difference between the average $\delta^{13}C_{\text{breath}}$ of bats of group A and B, and the animals of the fat turnover experiment (group E). To determine the expected asymptotic value of $\delta^{13}C_{\text{breath}}(\infty)$, we subtracted this difference from the mean $\delta^{13}C_{\text{breath}}$ of the animals of groups C and D. The expected $\delta^{13}C_{\text{breath}}(\infty)$ was used for estimating the other regression coefficient in the single-pool exponential model.

**Results**

**EQUILIBRATION OF STUDY ANIMALS TO TWO ISOTOPICALLY DISTINCT DIETS**

*Glossophaga soricina* that were maintained on a constant diet based on C$_4$/CAM plant products (C$_4$ bats) had a mean $\delta^{13}C_{\text{breath}}$ of $-11.8 \pm 1.2\%_o$ ($n = 24$) after having fasted over at least 5 h during the daytime. The baseline value of $\delta^{13}C_{\text{breath}}$ of unfed C$_4$ bats was not significantly different from the $\delta^{13}C$ of the C$_4$/CAM based diet (one sample Student’s $t$-test: $t_{33} = 0.93$, $P = 0.36$). Animals that were maintained on a diet based on C$_4$ plant products (C$_4$ bats) had a $\delta^{13}C_{\text{breath}}$ of $-26.5 \pm 1.2\%_o$ after having fasted over at least 5 h during the daytime. Similar to the stable carbon isotope signature of exhaled CO$_2$ in unfed C$_4$ bats, the $\delta^{13}C_{\text{breath}}$ of unfed C$_4$ bats was not significantly different to that of the their diet (one sample Student’s $t$-test: $t_{33} = 1.96$, $P = 0.062$). The difference in $\delta^{13}C_{\text{breath}}$ between C$_1$ and C$_4$ bats equalled 14.7$\%_o$ (Student’s $t$-test: $t_{43} = 43$, $P < 0.001$).

**FRACTIONAL INCORPORATION OF SUGARS INTO THE POOL OF METABOLIZED SUBSTRATE**

Baseline measurements of $\delta^{13}C_{\text{breath}}$ in unfed animals did not change during the 60 min of an experimental run, neither in unfed C$_3$ bats (repeated measures ANOVA: $F_{4,29} = 1.70$, $P = 0.67$) nor in unfed C$_4$ bats (repeated measures ANOVA: $F_{4,29} = 62$, $P = 0.08$). We estimated the fractional turnover of stable carbon isotopes in exhaled CO$_2$ of nectar-feeding bats after ingestion and combustion of different sugars, that is, fructose, glucose and sucrose (Fig. 1a–f). A few minutes after ingestion of naturally labelled sugar, the $\delta^{13}C_{\text{breath}}$ of the bats became enriched in $^{13}$C in C$_3$ bats and depleted in $^{13}$C in C$_4$ bats (Fig. 1). Equation coefficients for the single-pool exponential models of the six experiments are provided in Table 2 for each type of sugar. The average $t_{50}$ value for the fractional turnover of fructose was not significantly different between C$_1$ and C$_4$ bats (Student’s $t$-test: $t_{12} = 0.26$, $P = 0.80$) and equalled 8.7 ± 3.9 min. Similarly, average $t_{50}$ values were not significantly different between C$_1$ and C$_4$ bats for glucose (mean 12.8 ± 5.9 min; Student’s $t$-test: $t_{13} = 1.62$, $P = 0.13$) and sucrose (mean 14.0 ± 5.6 min; Student’s $t$-test: $t_{13} = 1.38$, $P = 0.19$). Fructose was turned over at the fastest rate followed by glucose and sucrose. We estimated the expected asymptotic plateau of the $\delta^{13}C_{\text{breath}}$ after bats were completely equilibrated to the stable carbon isotope signature of the recently ingested sugar water using a single-pool exponential model. The expected plateaus deviated in five of six cases from the dietary stable carbon isotope signature (Fig. 1). Only in C$_3$ glucose, $\delta^{13}C_{\text{breath}}$ was identical to the $\delta^{13}C$ of the sugar water.

**FAT TISSUE TURNOVER EXPERIMENT**

Bats in group E used for the fat tissue turnover experiment weighed on average 9.4 ± 0.8 g before the diet switch and 9.6 ± 0.7 g after the 4-day experiment. The change in body mass was not significant (paired $t$-test: $t_7 = 1.77$, $P = 0.16$). The average $\delta^{13}C_{\text{breath}}$ of bats of group E at day 0 equalled $-25.3 \pm 2\%_o$. After day 0, the $\delta^{13}C_{\text{breath}}$ of starved animals became enriched in $^{13}$C over the 4 days of the experiment, indicating the isotopic change of the diet from C$_3$ to C$_4$/CAM plant products (Fig. 2). We estimated a single-pool exponential regression equation for each individual bat and calculated a mean regression curve over all individuals.

The regression equation was: $\gamma(t) = -10.666 - 14.7\%_o \times (1-5\%_o)e^{-0.089 \times (t-123.9\text{days})}$ (regression coefficients given as mean ± 1 standard deviation). The fractional incorporation rate at which 50% of carbon atoms in fat tissue were exchanged by carbon atoms from dietary sugars equalled $t_{50} = 1.0 \pm 0.2$ days. Therefore, a carbon atom remains in the fat deposits of *G. soricina* for on average 1-4 days.
Use of exogenous substrates for metabolism

Fig. 1. $\delta^{13}$C$_{\text{breath}}$ (‰) in exhaled CO$_2$ of G. soricina that were fed a 30% (m/m) sugar solution of either fructose (a, $n = 8$; b, $n = 7$), glucose (c, $n = 8$; d, $n = 8$) or sucrose (e, $n = 8$; f, $n = 8$). Bats of the experiments shown in (a), (c) and (e) were first equilibrated to a diet derived from C$_3$ plants and then fed naturally labelled sugar water from C$_4$ or CAM plants and bats of the experiments shown in (b), (d) and (f) were first equilibrated to a diet derived from C$_4$ or CAM plants and then fed naturally labelled sugar water from C$_3$ plants. Solid circles represent mean values of $\delta^{13}$C$_{\text{breath}}$ for a given time interval since the bats were fed with sugar water for the first time. The T-mark depicts $\pm 1$ standard deviation, the dashed line the $\delta^{13}$C of the diet, and the curve exponential saturation curve fitted to the mean values. The $\Delta_{\text{diet-breath}}$ value indicates the mean difference between $\delta^{13}$C$_{\text{breath}}$($\infty$) and $\delta^{13}$C$_{\text{diet}}$ and $t_{50}$ the time required to exchange 50% of the carbon in exhaled CO$_2$ with those from recently ingested sugar.

Table 2. Types of sugars used in the fractional sugar incorporation experiments, and regression coefficients (mean $\pm$ 1 standard deviation) of the single-pool experimental model averaged across all individuals within each treatment group

<table>
<thead>
<tr>
<th>Sugar</th>
<th>$\delta^{13}$C$_{\text{breath}}$(‰)</th>
<th>$\delta^{13}$C$<em>{\text{breath}}$(0) $– \delta^{13}$C$</em>{\text{breath}}$(∞) (‰)</th>
<th>$k$ (days$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_3$ fructose</td>
<td>-22.73 ± 1.01</td>
<td>11.70 ± 1.28</td>
<td>0.0964 ± 0.0456</td>
</tr>
<tr>
<td>C$_4$ fructose</td>
<td>-12.47 ± 1.49</td>
<td>-14.43 ± 2.30</td>
<td>0.0955 ± 0.0420</td>
</tr>
<tr>
<td>C$_3$ glucose</td>
<td>-26.06 ± 1.48</td>
<td>14.66 ± 1.84</td>
<td>0.0952 ± 0.0570</td>
</tr>
<tr>
<td>C$_4$ glucose</td>
<td>-12.86 ± 1.24</td>
<td>-14.14 ± 1.89</td>
<td>0.0477 ± 0.0095</td>
</tr>
<tr>
<td>C$_3$ sucrose</td>
<td>-23.92 ± 1.38</td>
<td>11.83 ± 1.75</td>
<td>0.0652 ± 0.0222</td>
</tr>
<tr>
<td>C$_4$ sucrose</td>
<td>-16.74 ± 2.14</td>
<td>-9.70 ± 2.47</td>
<td>0.0538 ± 0.0331</td>
</tr>
</tbody>
</table>
Discussion

Fractional Incorporation Rates of Dietary Sugars into the Pool of Metabolized Substrates

Nectar-feeding *G. soricina* metabolized exogenous carbohydrates within minutes after feeding to sustain their high metabolic rate. The fractional incorporation of dietary sugars into the pool of combusted substrates was high in all three types of sugars, irrespective of whether they originated from C_3 or C_4/CAM plants. Most mammals studied thus far with respect to the rate of sugar ingestion and metabolism are much larger than the 10 g bats of our study, and since larger digestive tracts probably cause delays in the substrate intake, processing and combustion, the fast fractional turnover rates of dietary sugars in nectarivorous bats may be partly due to the relatively short time required to process the sugars in the alimentary tract. In mice, 40% of carbon isotopes were exchanged in exhaled CO_2 within 15 min when mice were fed corn (Perkins & Speakman 2001), suggesting that fractional turnover rates of exogenous substrate into the pool of metabolized substrates may be similarly high in other small mammals.

Hummingbirds make use of dietary carbohydrates at similarly fast rates. Welch *et al.* (2006) showed that starved broad-tailed hummingbirds (*S. platycerus*) fed with sucrose sustained 74% of their flight metabolism by sucrose combustion 20 min after drinking nectar. The fractional incorporation of dietary sugars into the pool of combusted substrates was slightly slower in *G. soricina* than in hummingbirds, although sucrase is abundantly present in both taxa (*G. soricina*: Hernandez & Martinez del Rio 1992; hummingbirds: Martinez del Rio & Karasov 1990; Martinez del Rio, Baker & Baker 1992). But given the threefold larger size of nectarivorous bats compared to hummingbirds, the mass-specific rates are even faster in nectarivorous bats than in hummingbirds. Yellow-rumped warblers (*Dendroica coronata*) that fed on a mixed diet of carbohydrates, proteins and fats, had mean t_50 values of 4.4 h (Podlesak, McWilliams & Hatch 2005), indicating that fractional incorporation rates of exogenous substrates into the pool of metabolized substrates may be slower when birds are dietary generalists or when feeding on a mixed diet instead of on a pure carbohydrate diet.

Monosaccharides such as fructose and glucose were metabolized at a similar rate as the disaccharide sucrose, probably since sucrase – the enzyme that hydrolyses the disaccharide into its monosaccharides – is abundantly present in the digestive tract of *G. soricina* (Hernandez & Martinez del Rio 1992). Previous preference tests in fruit-eating and nectar-feeding phyllostomid bats revealed that members of both feeding ensembles preferred sucrose to the monosaccharides glucose and fructose (Herrera 1999). This preference behaviour is not explained by sugar specific fractional incorporation rates at which bats allocated sucrose and hexose sugars into the pool of metabolized substrates.

Carbon atoms from naturally labelled glucose were not traceable in the δ^{13}C_{breath} before 30 min after sugar uptake in humans (Peronnet 2003) or even several hours after the meal in larger mammals such as horses (Ayliffe *et al.* 2004). Unfortunately, exact t_50 values cannot be calculated from data of Ayliffe and colleagues (2004) or Peronnet (2003) since the subjects did not even come close to the expected asymptotic plateau of equilibration. In horses, a maximum enrichment of ^{13}C in δ^{13}C_{breath} was reached after 5 h (4% enrichment of ^{13}C above baseline) which suggests that t_50 equals more than 5 h. In llamas t_50 equalled 2.8 h (Sponheimer *et al.* 2006). Summarizing, fractional incorporation rates of sugars into the pool of metabolized substrates of *G. soricina* are the fastest ever measured rates among mammals.

Combined Use of Exogenous and Endogenous Substrates

Non-exercising *G. soricina* metabolized exogenous carbohydrates immediately after ingestion and after an hour δ^{13}C_{breath} converged to the isotopic signature of the ingested sugars. Since δ^{13}C_{breath} was enriched in ^{13}C when animals fed sugars derived from C_3 plants but depleted when sugars originated from C_4 or CAM plants, and since differences caused by enzymatic fractionation should have the same sign for a given type of sugar, we conclude that differences between...
the asymptotic value of our regression equations and
the dietary δ^{13}C are most likely caused by fuelling
metabolism partly by endogenous substrate, such as
fat or glycogen. We calculated the proportion (P) at
which nectarivorous bats used endogenous substrates
by using a linear mixing model (eqn 3) for two pools
of metabolized substrates, that is, endogenous and
exogenous substrates:

\[
P = \left( \delta^{13}C_{\text{beats}}(\infty) - \delta^{13}C_{\text{carp}} \right) / \\
\left( \delta^{13}C_{\text{beats}}(0) - \delta^{13}C_{\text{carp}} \right) \text{ eqn 3}
\]

Bats used a significant portion of endogenous
substrate in addition to the oxidation of exogenous
carbohydrates. The mean proportion of endogenous
substrates in the pool of metabolized substrates
equalled 23 ± 17% for bats fed on sucrose water (one-
sample Student’s t-test: \( t_{14} = 6.6, P < 0.0001 \)), 18 ± 11% for
bats fed on fructose water (one-sample Student’s t-test:
\( t_{15} = 5.5, P < 0.0001 \)) and only 5 ± 10% for bats fed on
glucose water (one-sample Student’s t-test: \( t_{15} = 2.2, \\
P = 0.041 \)). Accordingly, nectarivorous bats used a
small, but significant proportion of endogenous
substrates after ingestion and digestion of a sugary diet.
Since enzymatic activity may follow a diurnal pattern
(Stevenson et al. 1975; Saito, Murakami, Suda 1976)
and since metabolism of dietary carbohydrates usually
occurs at night in free-ranging bats, it seems plausible
that nectarivorous bats fuel their metabolism completely
with exogenous substrate when foraging for nectar at
night. Most other mammals maintain their aerobic
metabolism predominantly by mobilising fat and
glycogen (Roberts et al. 1996); for example, endogenous
substrates support 70%–85% of the metabolic rate in
humans, with a higher contribution of exogenous
substrates in exercising subjects (Adopo et al. 1994;

Non-exercising hummingbirds, fuelled 90% of their
metabolism with exogenous carbohydrates (Carleton
et al. 2006), and exercising hummingbirds switch to
an almost exclusive combustion of exogenous
carbohydrates to maintain their costly flight activities
(Welch et al. 2006). Our findings in non-exercising
nectarivorous bats are in line with those from Carleton
et al. (2006), indicating a predominant but not exclusive
use of exogenous substrate when animals are resting.

TURN OVER OF FAT TISSUE IN VERTEBRATE
POLLINATORS

Glossophaga soricina turned over fat tissue 15 times
faster than, for example, Mongolian gerbils: \( t_{50} = 1.0 \)
days in nectarivorous bats compared to \( t_{50} = 15.6 \)
days in gerbils, but turnover rates of fat tissues were almost
equal to those reported for hummingbirds (\( t_{50} = 1.5 \)
and 0-8 days in broad-tailed hummingbirds; Carleton
et al. 2006). This high turnover of fat stores in both
pollinator taxa is most likely caused by their relatively
small fat deposits and their relatively high mass-specific
metabolic rate (Voigt et al. 2006) and it underlines that
nectar specialists must balance lipogenesis and fat
catabolism very accurately if they are to remain in
energy balance.

ALLOCATION OF SUBSTRATES TO
METABOLISM, STORAGE AND TISSUE
SYNTHESIS

This study suggests the hypothesis that ingested carbo-
hydrates may be allocated preferentially to metabolism
and not to tissue synthesis or storage. Consequently,
stable carbon isotopes of the carbohydrate portion of
a diet is detectable in the breath of an animal, but not
necessarily in the solid tissue in which mostly proteins
or fat are incorporated (Perkins & Speakman 2001).
Previous isotopic studies in G. soricina showed that the
\( t_{50} \) values of stable carbon isotopes was low for red
blood cells when bats were sustained on a carbohydrate
rich/low protein diet (Voigt & Matt 2002; Voigt et al.
2003) and that estimates of \( t_{50} \) increased with decreasing
percentage of carbohydrates and increasing percentage
of protein in the diet (Miron et al. 2006). In light of the
current breath isotope study, it becomes clear that bats
mostly metabolized the few available carbohydrates in
the study by Miron et al. (2006), whereas carbohydrates
were incorporated into blood cells in the study by Voigt
et al. (2003). Therefore, the contrasting difference of \( t_{50} \)
values in the range of 20–40 days in Miron et al. (2006)
and more than 100 days in Voigt et al. (2003) for blood
cells most likely resulted from different enzymatic
pathways bats used. Bats that were sustained on a
carbohydrate poor diet mostly metabolized sugars and
assimilated proteins, whereas bats that were sustained
on a carbohydrate rich diet metabolized carbohydrates,
but in addition assimilated carbohydrates also into
blood cells. Following this line of argument, a carbo-
hydrate rich diet of free-ranging nectarivorous bats
would cause a low and a protein-rich diet a fast
turnover rate of blood cells.

THE ECOLOGICAL CONTEXT OF SUBSTRATE
USE

Nectar specialists live in the fast lane having metabolic
rates higher than those of similar-sized animals with
different feeding habits (Voigt et al. 2006). The
underlying cause for this phenomenon is that nectar
specialists are forced by flowering plants to continu-
ously search for new nectar sources since nectar
rewards are barely large enough to satisfy the energetic
needs of pollinator-specific flower exploitation costs,
making flower visitation marginally profitable, but
sufficiently small to encourage pollinators to return to
the flowers after nectar replenished (Klinkhammer &
deJong 1993; Voigt et al. 2006). Since aerial locomotion
is more costly for fat than for lean individuals (Voigt
2000), fat deposits should be regulated so that they
are able to supply the energy requirements of diurnal
inactivity, that is, in times when exogenous substrates

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are absent. Carbohydrates derived from flowers may be the more appropriate substrate for fuelling the pollinator’s activities when foraging for nectar. This raises the question of whether fat deposits are turned over at sufficient rates to support the metabolism of resting nectarivorous bats in the daytime roost.

The body mass of free-ranging G. soricina contains c. 5% fat stores (McNab 1976). A $t_{50}$ value of 1–0 days for fat tissue turnover suggests that 50% of body fat is exchanged over a day. Consequently, a 9·6-g nectarivorous bat mobilizes c. 0·24 g fat each day. Assuming that the energy equivalent of 1 g fat is 39 kJ (Wieser 1986), bats would derive 9·4 kJ of energy each day by catalyzing lipids. Given a field metabolic rate of 45·7 kJ d$^{-1}$ for a glosophagine bat of similar mass (Voigt et al. 2006), 21% of energy turned over per day originates from the mobilization of fat stores. According to Cruz-Neto & Abe (1997), resting metabolic rate of our G. soricina equals 65·6 mLO$_2$ h$^{-1}$ at 25 °C. This adds up to 787 mL O$_2$ for the 12 h period of daylight. Converting this value into an energy equivalent (19·81 J mL O$_2$ at RQ 0·75; Wieser 1986) yields 15·6 kJ of energy turned over during the daytime. The fact that fat contributes only with 9·4 kJ to the daytime metabolism supports the observation by Kelm & von Helversen (2007) that bats use a mixture of fat and carbohydrates during the beginning of day and then increasingly metabolize endogenous substrate. This would, however, require that the nocturnal life of nectarivorous bats is completely or predominantly fuelled by exogenous carbohydrates, yielding a slight surplus by the end of the night. The current study suggests that nectarivorous bats mostly, if not exclusively, use exogenous sugars when available to fuel their metabolically expensive activities at night.

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Use of exogenous substrates for metabolism


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