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Fruit bats (Pteropodidae) fuel their metabolism rapidly and directly with exogenous sugars

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SUMMARY

Previous studies reported that fed bats and birds mostly use recently acquired exogenous nutrients as fuel for flight, rather than endogenous fuels, such as lipids or glycogen. However, this pattern of fuel use may be a simple size-related phenomenon because, to date, only small birds and bats have been studied with respect to the origin of metabolized fuel, and because small animals carry relatively small energy reserves, considering their high mass-specific metabolic rate. We hypothesized that ~150 g Egyptian fruit bats (*Rousettus aegyptiacus* Pteropodidae), which are more than an order of magnitude heavier than previously studied bats, also catabolize dietary sugars directly and exclusively to fuel both rest and flight metabolism. We based our expectation on the observation that these animals rapidly transport ingested dietary sugars, which are absorbed *via* passive paracellular pathways in the intestine, to organs of high energy demand. We used the stable carbon isotope ratio in exhaled CO_2 ($\delta^{13}C_{breath}$) to assess the origin of metabolized substrates in 16 Egyptian fruit bats that were maintained on a diet of C3 plants before experiments. First, we predicted that in resting bats $\delta^{13}C_{breath}$ remains constant when bats ingest C3 sucrose, but increases and converges on the dietary isotopic signature when C4 sucrose and C4 glucose are ingested. Second, if flying fruit bats use exogenous nutrients exclusively to fuel flight, we predicted that $\delta^{13}C_{breath}$ of flying bats would converge on the isotopic signature of the C4 sucrose they were fed. Both resting and flying Egyptian fruit bats, indeed, directly fuelled their metabolism with freshly ingested exogenous substrates. The rate at which the fruit bats oxidized dietary sugars was as fast as in 10 g nectar-feeding bats and 5 g hummingbirds. Our results support the notion that flying bats, irrespective of their size, catabolize dietary sugars directly, and possibly exclusively, to fuel flight.

Key words: flight, metabolic rate, stable isotopes, Chiroptera.

INTRODUCTION

Although flight is the most economical mode of animal locomotion in terms of energy spent per unit distance traveled, it necessitates a much higher energy cost per unit time than any other form of locomotion, making flapping flight the most immediately expensive means of locomotion (Schmidt-Nielsen, 1997; Masman and Klaassen, 1987; McNeill, 2002). In general, energy turnover during flapping flight exceeds basal metabolic rate by a factor of 8–15 in vertebrates (Lasiewski, 1963; Tucker, 1968; Speakman and Thomas, 2003).

Flying animals should be particularly careful about their choice of fuels because of the high cost of flight, particularly when carrying surplus body mass (e.g. Hambly et al., 2004) (but see Kvist et al., 2001). Thus, although fat reserves may be efficient with respect to energy density, they may increase flight costs enormously (Voigt, 2000). The fuel(s) chosen by an animal depends mostly on its nutritional status and its particular activity. During endurance migratory flights, for example, birds usually do not feed on the wing and, thus, cannot use exogenous substrates. Instead, they depend exclusively on endogenous sources of fuel, i.e. they support almost all of their energy expenditure by oxidizing fatty acids from adipose cells (Jenni and Jenni-Eiermann, 1998; Jenni-Eiermann et al., 2002; McWilliams et al., 2004; Schaub et al., 2008). In contrast, non-migratory nectar-feeding birds oxidize mostly exogenous sugars when active (Suarez et al., 1990; Welch et al., 2006; Welch et al., 2008). These two examples lie at opposite ends of a continuum between the use of exogenous sugars and endogenous fat. Other fuel choices are possible as well. For example, short and intermediate bouts of flight are fuelled almost exclusively by combustion of endogenous sugars (e.g. glycogen) in birds (Rothe et al., 1987). In this light, we addressed the question: what kind of fuels do large bats use during flight, given that their energy demands are much higher than those of similar-sized exercising, non-volant mammals? In addition, we asked: do these fuels derive from exogenous or endogenous sources?

Previous studies emphasized that fed nectarivorous bats fuel their very high mass-specific metabolism exclusively with exogenous carbohydrates (Voigt and Speakman, 2007; Welch et al., 2008). However, fuel choice and origin may be a function of animal size. Large animals can store relatively more fat than small animals (Schmidt-Nielsen, 1997), and all bats studied to date with respect to the origin of oxidized fuels weighed less than 20 g. Therefore, we investigated the origin of the fuels used by active and inactive ~150 g Egyptian fruit bats, *Rousettus aegyptiacus* Geoffroy 1810, a species which is an order of magnitude heavier than the birds and bats studied so far with respect to the origin of metabolic fuel.

The diet of free-ranging Egyptian fruit bats consists mostly of fruits and leaves (Korine et al., 1999a). As an adaptation to a carbohydrate-rich diet, *R. aegyptiacus* is able to absorb 95% of all sugars ingested during the first 45 min after a fruit meal (Keegan et al., 1977). In addition, Tracy and colleagues (Tracy et al., 2007) found that at least 55% of the glucose absorbed in the intestine of *R. aegyptiacus* is passively transported *via* the paracellular pathway, i.e. through spaces between the cells and not through the enterocytes. Thus, it is likely that exogenous carbohydrates are rapidly available for use by the locomotory muscles of *R. aegyptiacus*, despite the relatively large body mass (m_b) of these bats.

In the present study, we measured the stable carbon isotope ratio of exhaled breath ($\delta^{13}C_{breath}$) to discern the origin of oxidized fuel in active and inactive *R. aegyptiacus*. $\delta^{13}C$ represents the ratio of $^{13}C/^{12}C$ of the sample in relation to the ratio in an international standard in parts per mille (‰) (reviewed in Fry, 2006; Martínez del Rio et al., 2009). Our approach was based on the premises that (1) $\delta^{13}C_{breath}$ matches $\delta^{13}C$ of the fuel being oxidized (e.g. Schoeller et al., 1984) (reviewed in Voigt et al., 2008c; Engels et al., 2009), and that (2) if $\delta^{13}C$ of the new diet contrasts with $\delta^{13}C$ of the maintenance diet, $\delta^{13}C_{breath}$ of post-absorptive bats should converge on $\delta^{13}C$ of the new diet according to the relative contribution of exogenous fuels to metabolism (Perkins and Speakman, 2001; Hatch et al., 2002a; Hatch et al., 2002b). During the summer, the maintenance diet of our captive colony of *R. aegyptiacus* consisted of melon fruit (*Citrullus* and *Cucumis* sp.), which are cucurbit plants whose photosynthetic CO_2 incorporation follows the C3 metabolic pathway (Markow et al., 2001), whereas during feeding experiments bats were fed products of plants (glucose or fructose) that followed the C4 metabolic pathway. Accordingly, the maintenance and experimental diet differed by about 14‰ in $\delta^{13}C$.

We hypothesized that large bats such as *R. aegyptiacus* would employ the same strategy of energy source use as small bats. We expected that they would fuel all their metabolic requirements by catabolizing exogenous carbohydrates, both at rest and at flight. Accordingly, we predicted that $\delta^{13}C_{breath}$ of resting post-adsorptive *R. aegyptiacus* would converge on $\delta^{13}C$ of C4 sugars when bats were fed the experimental diet, whereas $\delta^{13}C_{breath}$ of resting post-adsorptive *R. aegyptiacus* would remain constant when bats were fed C3 sugars. For active *R. aegyptiacus* we predicted that $\delta^{13}C_{breath}$ would also level off at the isotopic signature of the newly ingested diet after the animals fed on C4 sucrose because the rapid paracellular transport of sugars (Tracy et al., 2007) facilitates the use of exogenous sugars, even when energy demands are considerably elevated.

MATERIALS AND METHODS

Metabolic substrate use in resting *R. aegyptiacus*

We studied the origin of metabolized substrates in eight adult male *R. aegyptiacus* in July 2008. The bats were held in captivity for 5 years in facilities of the Blaustein Institutes for Desert Research on the Sede Boqer Campus of Ben-Gurion University, Israel, in a shaded outdoor flight-cage (area 45 m^2; height 2.5 m), under natural dark–light conditions. Each day, animals were fed a diet of watermelons (*Citrullus lanatus*) and honeydew melons (*Cucumis melo*). In general, the carbohydrate concentration of melon is about 80% dry matter which is similar to the average carbohydrate concentration (80.5±5.85% dry matter) in fruit eaten by free-ranging Egyptian fruit bats (Korine et al., 1988). Feeding times matched the animals' natural diurnal pattern of food intake. Ambient temperature in the flight-cage averaged 28°C and the natural photoperiod was 13 h (light):11 h (dark). Bats were distinguished by individual ear-tags. This research was done under permit 18150 from Israel Nature and National Parks Protection Authority.

Each of the eight bats was used in three feeding trials in random order, with at least a 2 day intermission between successive trials. Before trials, m_b of bats was measured to ±1 g using a digital balance (Scout Model SC2020, Ohaus Corp., Florham Park, NJ, USA). We fed the bats aqueous solutions containing one of three sugars: glucose from C4 plants, sucrose from C4 plants or sucrose from C3 plants. We collected samples of each sugar fed during the diet-switch (see below) experiments and from the bats' normal diet for later analysis using isotope ratio mass spectrometry (IRMS). Samples were dried in a drying oven at 40°C to constant mass, weighed on a Sartorius microbalance (Sartorius AG, Göttingen, Germany) and loaded in tin capsules. All samples were burned and analyzed with a Flash Elemental Analyzer and a Conflo II, coupled to a Delta-Advantage IRMS (Delta V Advantage, FischerThermo, Bremen, Germany) at the Stable Isotope Laboratory of the Leibniz Institute for Zoo and Wildlife Research Berlin (IZW), Germany. Samples were analyzed in combination with internal standards that had previously been calibrated against an international ^{13}C standard (NBS 22). All $^{13}C/^{12}C$ were expressed relative to the international standard in the δ notation (‰) using the following equation:

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

with $^{13}C/^{12}C$ representing the isotope ratio in either the sample or the standard. Precision was better than ±0.03‰ (1σ). Stable carbon isotope ratios of dietary items are listed in Table 1.

The bats were administered sugar solutions with concentrations accurately prepared using an electronic balance (Presica 40SM-200A, accuracy ±0.0001 g; Bradford, UK). All experiments lasted for 90 min and were performed at night during the natural activity period of the bats. Before feeding bats with the sugar solutions, we collected an initial breath sample from each individual. Following the ingestion of the first solution we collected samples at intervals ranging from 3 to 12 min, with most being ~7 min. For breath sampling, bats were transferred to individual cotton bags (length 30 cm, width 20 cm) that were each placed in a larger plastic bag (length 28 cm, 28 cm width; Ziplock™, Racine, WI, USA). Ambient air, scrubbed of CO_2 using NaOH (JT Baker, Deventer, Netherlands), was flushed through the bag *via* a plastic entry tube (diameter 3 mm) at a flow rate of 850 ml min^{-1} (STP). The flushing gas exited the plastic bag through a 2–4 cm slit (0.2 cm wide). A small PE tube was positioned with one end close to the bat's head inside the bag (diameter 1 mm, length 4 cm). We let CO_2 accumulate by stopping air circulation for 1.5 min. Adult *R. aegyptiacus* have a resting O_2 uptake ($\dot{V}_{O_2}$) of approximately 123 ml min^{-1} (Noll, 1979). Therefore, we expected CO_2 to accumulate to about 3% within 1 or 2 min. We then drew air, including the bat's breath, from the bag into an infrared stable isotope analyzer (IR-SIA; HelifanPlus, Fischer GmbH, Leipzig, Germany). Routine checks of the instrument's accuracy

Table 1. Stable carbon isotope ratios (‰) in the maintenance diet (melon based) and in sugars of different origins fed to *Rousettus aegyptiacus*

Diet	Resting bats	Flying bats
Melon (C3)	–26.3±0.5‰	
Sucrose (C4)	–12.0±0.1‰	–12.2±0.1‰
Sucrose (C3)	–25.5±0.2‰	Not offered
Glucose (C4)	–10.4±0.1‰	Not offered

shortly before the experiment revealed no significant deviation from expected $\delta^{13}C_{breath}$ measured using conventional IRMS (Voigt, 2009).

After breath collection, we restarted the air circulation in the plastic bag. Since we expected the transition from catabolism of endogenous substrates to catabolism of exogenous substrates to be completed within 60 min of the initial sugar meal (Voigt and Speakman, 2007; Voigt et al., 2008a; Voigt et al., 2008b), we continued breath collection from a given animal for a total of 90 min. Each bat was fed three times with 7–8 ml of sugar solution at times 0, 30 and 60 min. The IR-SIA measured the adsorption of infra-red light, which depends on the ratio between $^{13}CO_2$ and $^{12}CO_2$ in the gas sample. All ^{13}C:^{12}C ratios were expressed relative to the international standard in the δ notation (‰). We defined isotopic equilibration of an animal's tissue or breath as the status where the isotopic composition of animal tissue or breath reached a plateau and remained constant while the animal continued to consume the same food (in our case, sugar solutions).

Metabolic substrate use in flying *R. aegyptiacus*

Experiments with flying bats were done in August 2009 with 8 adult male *R. aegyptiacus* that were captured 7 days before use in Beer Sheba, Israel. Bats in this group were individually labeled on a wing with an indelible marker. Before each trial, we measured m_b of the bats to ±0.1 g using a digital balance (Scout Model SC2020, Ohaus Corp.). During trials, non-participating bats were temporarily held in small cages while participating bats were allowed to fly freely in the same flight cage as described above. Trials lasted for an average of 102±10 min (mean ± s.d.), of which the bats flew for an average of 30 min. During the trial, bats were encouraged to fly as much as possible by two persons who gently removed them from the walls or ceiling when they landed and released them to continue flying. Flight experiments began at the onset of the animals' natural activity period, shortly after sunset at around 19:00 h, and lasted, at the latest, until 22:00 h.

We measured $\delta^{13}C_{breath}$ following an experimental protocol similar to that used in trials with resting *R. aegyptiacus* in 2008, but modified as follows. First, instead of measuring the $\delta^{13}C_{breath}$ on site, we collected breath samples using two 100 ml gas-tight glass syringes that were sealed and brought to the laboratory in which the analyzer was located. On average, less than 15 min elapsed between breath collection and measurement. Second, we collected breath samples less often than during tests with resting bats, to let the bats fly as much as possible. Thus, the interval between breath collection averaged 21.0±0.7 min (mean ± s.d.). Third, we used only C4 sucrose for the diet-switch experiment, because sucrose is the dominant sugar in the fruits eaten by Old World fruit bats (Baker et al., 1998) and because the trials with resting bats revealed no significant difference between the rates of catabolism of monosaccharides and disaccharides. Finally, we offered bats a 30% (mass/mass) sugar/water solution to ensure that they ingested sufficient energy to cover the metabolic requirements of flight during the experiments. We calculated the minimum amount of sugar necessary to keep them in positive balance by estimating their energy expenditure during a trial. We multiplied the mass-specific $\dot{V}_{O_2}$ of *R. aegyptiacus* at an ambient temperature of 32°C ($\dot{V}_{O_2}$=0.95±0.15 ml g^{-1} h^{-1}) (Korine and Arad, 1993) by m_b (150±9 g) and converted the total amount of oxygen consumed into metabolic rate (MR) by using the energetic equivalent of sugar combustion. Thus, the mean MR of *R. aegyptiacus* was 0.79 W. We then used the Speakman and Thomas (Speakman and Thomas, 2003) allometric equation for flight costs in bats to estimate the total energy turnover of a 150 g bat flying for 30 min during a flight trial. Combining these two estimates, we expected bats would use 26.5 kJ during a flight trial. By raising the concentration of sugar in solution to 30%, we provided bats with a total of 119 kJ – equivalent to 7.2 g of sucrose with an energy value of approximately 16 kJ g^{-1} (Levin et al., 1995), well above the estimated energy needs of the bats.

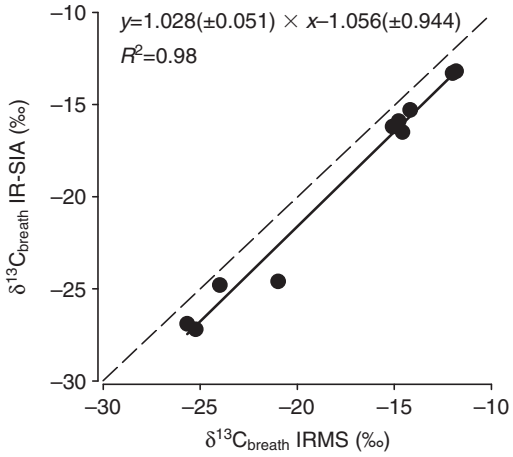

Fig. 1. Stable carbon isotope ratios of exhaled breath ($\delta^{13}C_{breath}$; ‰) samples measured with both an infra-red stable isotope analyzer (IR-SIA) and a conventional isotope ratio mass spectrometer (IRMS). Breath samples originated from *Rousettus aegyptiacus* fed solutions with varying proportions of C3 and C4 sugars. The dashed line indicates the line of equivalence and the solid line a least-squares, linear regression equation that best describes the data.

To assess the accuracy of the IR-SIA we conducted a validation experiment similar to that described by Voigt (Voigt, 2009), feeding animals diets of various proportions of the C3 and C4 solutions to yield $\delta^{13}C_{breath}$ values that varied over the expected range between sugars of C3 and C4 plant origin. Instead of measuring $\delta^{13}C_{breath}$ only with the IR-SIA, we collected a second sample using an evacuated glass vial (Vacutainer®; Labco, High Wycombe, Bucks, UK). For this we placed one end of a small plastic tube (2 mm in diameter) in the tube ending that collected the breath samples for the IR-SIA. The other end was attached to a syringe. At the same time that $\delta^{13}C_{breath}$ was measured, we pierced the Teflon membrane of the Vacutainer with the needle and allowed it to fill with the animal's expirate. The filled Vacutainers were brought to the Stable Isotope Laboratory of the IZW, where $\delta^{13}C_{breath}$ was measured using a GasBench coupled to an IRMS at the stable isotope laboratory (see above). We plotted $\delta^{13}C_{breath}$ measured with IR-SIA in relation to $\delta^{13}C_{breath}$ measured with IRMS and calculated a least-squares linear regression equation (Fig. 1). The slope of this regression was not different from 1 (Student's *t*-test; t_9=0.55; P>0.05) and the *y*-intercept was not different from 0 (Student's *t*-test; t_9=1.12; P>0.05). Thus, we consider the IR-SIA sufficiently accurate to yield true $\delta^{13}C_{breath}$ values; a finding that is supported by the measurements of the previous year (Voigt, 2009).

Fractional incorporation of dietary sugars into the pool of metabolized substrate

To monitor the dynamics of metabolic substrate use in resting and flying animals, we used a one-pool model, since previous measurements indicated that carbohydrate oxidation follows a one-pool, not a two-pool, model in bats (Voigt and Speakman, 2007;

Voigt et al., 2008a; Voigt et al., 2008b; Voigt et al., 2008c). For each bat we used the following equation:

$$\delta^{13}C_{breath}(t) = \delta^{13}C_{breath}(\infty) + [\delta^{13}C_{breath}(0) - \delta^{13}C_{breath}(\infty)] \times e^{-tk}, \quad (2)$$

where $\delta^{13}C_{breath}(t)$ is the stable carbon isotope ratio of exhaled CO_2 at time t; $\delta^{13}C_{breath}(\infty)$ is the asymptotic stable carbon isotope ratio of exhaled CO_2 when animals are equilibrated to the stable carbon isotope signature of their diet; $\delta^{13}C_{breath}(0)$ is the stable isotope ratio of exhaled CO_2 at time zero of the experiment; and k is the fractional turnaround time of carbon atoms in the pool of metabolized substrate (min^{-1}). k was estimated iteratively using Systat® (version 11.00.01, 2004; Systat Software Inc., San José, CA, USA). We averaged the regression coefficients of all individuals to derive a regression equation for each trial with a certain sugar type and calculated the time at which 50% of carbon isotopes were exchanged in the animals' breath (t_{50}) according to the following equation: t_{50}=–ln(0.5)/k, where 0.5 is the exchange of 50% of the isotope. We used Student's t-test to test for differences in mean $\delta^{13}C_{breath}(\infty)$ and the stable carbon isotope ratio of the sugar solution, after ascertaining that the data were normally distributed and homoscedastic. All values are presented as means ± s.d. and all statistical tests were two-tailed if not stated otherwise.

RESULTS

Metabolic substrate use in resting *R. aegyptiacus*

Oxidation of C4 glucose

Body mass of *R. aegyptiacus*, measured before feeding at between 20:00 and 21:00 h averaged 146.9±4.5 g (N=8). The $\delta^{13}C_{breath}$ of fasted *R. aegyptiacus* averaged –25.9±1.7‰ which is not significantly different from $\delta^{13}C$ of the melon diet (–26.3±0.5‰) that bats fed on during the period preceding breath collection experiments (Student's t-test: t_7=0.73; P=0.49). After feeding bats with C4 glucose ($\delta^{13}C$=–10.4±0.1‰), exhaled breath became enriched in $^{13}CO_2$ (Fig. 2A) and after 90 min, $\delta^{13}C_{breath}$ reached an asymptote at –11.5±1.9‰, which was not different from the dietary $\delta^{13}C$ (t_7=1.69; P=0.134). For C4 glucose, the regression equation describing the fractional incorporation rate of carbon atoms into the pool of metabolized substrate is: $\delta^{13}C_{breath}(t)$=–11.5–15.1$e^{-0.07t}$ (Table 2). t_{50} for the exchange of carbon atoms of the metabolic fuel with those of dietary glucose averaged 10.7±2.9 min (Table 2).

Oxidation of C4 sucrose

After bats were fed a solution containing C4 sucrose ($\delta^{13}C$=–12.0±0.1‰), exhaled breath became enriched in $^{13}CO_2$ (Fig. 2B). The regression equation for C4 sucrose is: $\delta^{13}C_{breath}(t)$=–10.9–15.3$e^{-0.05t}$. $\delta^{13}C_{breath}$ leveled off at –10.9±1.6‰ after 90 min, which was not significantly different from the $\delta^{13}C$ of the ingested sugar (t_7=1.85; P=0.11). t_{50} for exchange of carbon atoms of the metabolic fuel with those of dietary sucrose was 13.6±3.2 min which is not significantly different from the t_{50} of glucose catabolism (paired t-test; t_7=1.61; P=0.15).

Oxidation of C3 sucrose

For logistic reasons, we included only seven out of eight individuals in this experiment, and, due to a technical failure we were not able to obtain breath data from two individuals at the end of the experiment. Therefore, we used repeated measures ANOVA to analyze data from seven bats, over 85 min of the experiment, and found that $\delta^{13}C_{breath}$ did not change over the course of 90 min following ingestion of sucrose from C3 plants ($\delta^{13}C$=–25.5±0.2‰; repeated measures ANOVA: $F_{11,83}$=1.48, P=0.16; Fig. 2C). The mean $\delta^{13}C_{breath}$ equaled –25.3±1.6‰, which is not significantly different from the isotopic signature of the diet (t_7=1.91; P=0.098; Table 2).

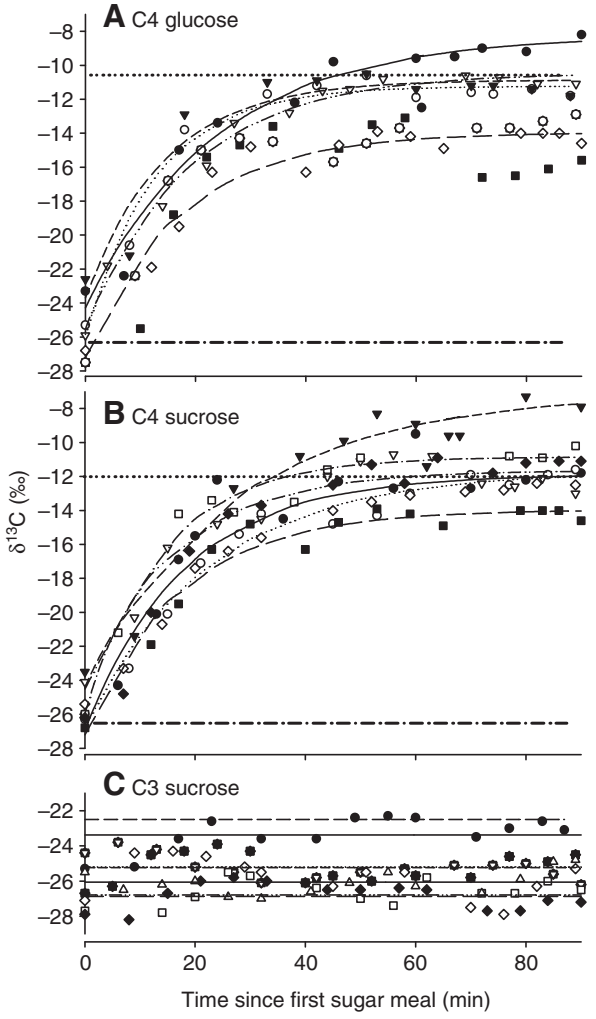

Fig. 2. Stable carbon isotope ratio of exhaled breath ($\delta^{13}C_{breath}$; ‰) in eight resting *R. aegyptiacus* after they were fed with sugar solutions that consisted of glucose from C4 plants (A) or of sucrose from either C4 or C3 plants (B,C). Before the experiments bats were maintained on a diet of melons with a stable carbon isotope signature of –26.3±0.5‰. Dotted straight lines indicate the isotope signature of the respective sugar and dashed-dotted lines those of the maintenance diet. Symbols and lines depict individual animals.

Metabolic substrate use in flying *R. aegyptiacus*

Oxidation of C4 sucrose

Before being fed a sugar solution, *R. aegyptiacus* in group 2 weighed on average 149.7±9.4 g (N=8). Fasted *R. aegyptiacus* had an average $\delta^{13}C_{breath}$ of –24.4±1.4‰, which plateaued at –12.4±1.6‰ after ingestion of a sugar solution containing C4 sucrose (Fig. 3). The regression equation for C4 sucrose is: $\delta^{13}C_{breath}(t)$=–12.4+12.0$e^{-0.07t}$ (Table 3). t_{50} for exchange of carbon atoms in the pool of metabolized substrate with exogenous carbon atoms from the sugar water equaled 10.9±5.8 min (Table 3), which is not different from the mean t_{50} obtained from resting bats feeding on C4 sucrose (t_7=1.15; P=0.72).

Table 2. Individual values for the fractional incorporation rate of ingested sugars into the pool of metabolized substrates during rest for C_4 glucose and C_4 sucrose for 8 *R. aegyptiacus*

	C4 glucose					C4 sucrose					C3 sucrose	
Individual	*a* (‰)	*b* (‰)	*k* (min^{-1})	t_{50} (min)	R^2	*a* (‰)	*b* (‰)	*k* (min^{-1})	t_{50} (min)	R^2	$\delta^{13}C_{breath}$ (‰)	r_s
1	–8.2	–16.1	–0.04	16.5	0.996	–11.9	–15.0	–0.06	12.6	0.968	–23.4	0.37
2	–11.2	–14.6	–0.08	8.8	0.997	–11.7	–15.1	–0.04	15.8	0.974	–25.4	–0.49
3	–10.9	–12.8	–0.07	9.9	0.992	– 7.1	–17.3	–0.04	18.7	0.968	–25.2	0.14
4	–10.5	–15.1	–0.05	13.1	0.988	–12.0	–13.0	–0.06	11.6	0.921	–26.8	0.45
5	–12.9	–15.8	–0.08	8.7	0.731	–10,8	–15.2	–0.07	9.9	0.975	–26.9	–0.07
6	–13.6	–14.3	–0.08	8.5	0.948	–11.0	–17.0	–0.05	13.6	0.969	–25.8	–0.46
7	–11.0	–19.0	–0.08	8.6	0.979	–12.0	–14.0	–0.04	16.9	0.989	–22.5	–0.39
8	–14.0	–13.0	–0.06	12.0	0.956	–11.0	–16.0	0	10.0	0.948	–26.0	0.35
Mean	–11.5	–15.1	–0.07	10.7		–10.9	–15.3	–0.05	13.6		–25.3	
±1 s.d.	±1.9	±1.9	±0.15	±2.9		±1.6	±1.5	±0.01	±3.2		±1.6	

Data calculated with a single-pool exponential regression model ($y=a+b\times e^{-tk}$) and $\delta^{13}C_{breath}$ (‰) for C_3 sucrose averaged over all data points. The regression parameter *a* represents $\delta^{13}C_{breath}(\infty)$, *b* represents $[\delta^{13}C_{breath}(0)–\delta^{13}C_{breath}(\infty)]$ and *k* represents the fractional turnaround time of carbon atoms in the pool of metabolized substrates. t_{50} equals the time at which 50% of carbon atoms were exchanged in the animals' breath, R^2 the level of determination and r_S the Spearman rank correlation coefficient.

DISCUSSION

Metabolic substrate use in resting *R. aegyptiacus*

Our results indicate that resting *R. aegyptiacus* catabolize endogenous fuels when fasting and mostly exogenous carbohydrates when food is available. In fasting animals, $\delta^{13}C_{breath}$ values were similar to the C3 isotopic signature of their maintenance diet, indicating that bats used endogenous fuels (lipids and glycogen). After having ingested the C3 sugar solution, $\delta^{13}C_{breath}$ remained constant throughout the experiment, whereas $\delta^{13}C_{breath}$ increased after bats ingested a C4 sugar solution. Thus, $\delta^{13}C_{breath}$ always converged on the isotopic value of the ingested sugars. If bats had metabolized a combination of endogenous fuels, such as lipids or glycogen, and exogenous sugars, we would have expected to find intermediate $\delta^{13}C_{breath}$ values, reflecting the relative contributions of endogenous energy sources with a C3 isotope signature and exogenous sources with a C4 isotope signature to the metabolism of bats. Indeed, some individuals exhibited intermediate values during the plateau phase, but we attribute these to instability in the measurements given that overestimation and underestimation of the expected plateau value occur to the same extent. In general, our results are in accord with those of Korine and colleagues (Korine et al., 1999b) who found that blood glucose and triglyceride levels are negatively correlated in postprandial Egyptian fruit bats indicating that exogenous glucose is the prime energy source during their active phase at night, and endogenous lipids are the prime energy source during their daytime rest phase.

The exponential rise in $\delta^{13}C$ values, coupled with a short t_{50}, indicates a very rapid shift from endogenous to exogenous fuels as soon as bats ingested the experimental diet. The incorporation rates of exogenous sugars into the pool of metabolized substrates was similar for the two C4 sugars (t_{50}=11 min for glucose and 14 min for sucrose), even though sucrose is the larger molecule, and thus could potentially be transferred from gut to blood less efficiently by means of passive paracellular transport in *R. aegyptiacus* (Tracy et al., 2007). The rate at which *R. aegyptiacus* used exogenous carbon as a fuel – i.e. the speed at which bats changed from lipid and glycogen combustion to the catabolism of exogenous sugars, was as fast as in 10 g nectar-feeding bats, 20 g fruit-eating bats or 5 g hummingbirds (Voigt and Speakman, 2007; Voigt et al., 2008b; Welch et al., 2008), and faster than in bats with a protein-dominated diet, such as

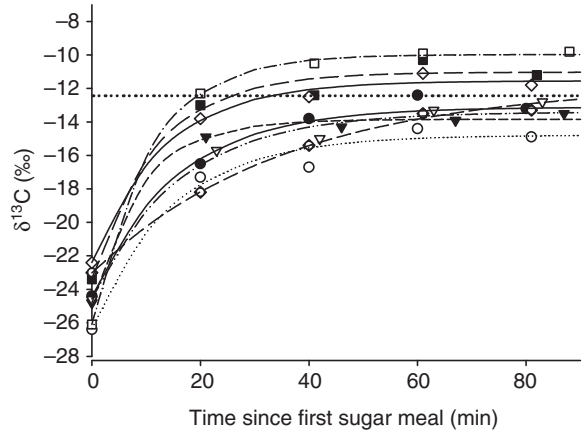

Fig. 3. Stable carbon isotope ratio of exhaled breath ($\delta^{13}C_{breath}$; ‰) in eight flying *R. aegyptiacus* after they were fed a sugar solution that consisted of sucrose from a C4 plant. Before the experiments bats were maintained on a diet of melons with a stable carbon isotope signature of *ca.* –26.3±0.5‰. The dotted straight line indicates the isotope signature of sugar and the dashed-dotted line that of the maintenance diet. Symbols and lines depict individual animals.

Table 3. Variables for the fractional incorporation rate of ingested sugars into the pool of metabolized substrates during exercise for C_4 sucrose for 8 *R. aegyptiacus*

Individual	*a* (‰)	*b* (‰)	*k* (min^{-1})	t_{50} (min)	R^2
1	–11.0	12.4	–0.09	8.1	0.982
2	–13.1	11.3	–0.07	10.7	0.982
3	–14.8	11.6	–0.07	10.2	0.997
4	–13.8	11.0	–0.11	6.4	0.984
5	–13.4	11.2	–0.06	11.0	0.987
6	–10.0	16.1	–0.10	7.2	0.999
7	–11.7	11.3	–0.03	24.8	0.972
8	–11.5	10.8	–0.08	9.1	0.994
Mean ± 1 s.d.	–12.4±1.6	12.0±1.7	–0.07±0.02	10.9±5.8	

Data calculated with the single-pool exponential regression model ($y=a+b\times e^{-tk}$). The regression parameter *a* represents $\delta^{13}C_{breath}(\infty)$, *b* represents $[\delta^{13}C_{breath}(0)–\delta^{13}C_{breath}(\infty)]$ and *k* represents the fractional turnaround time of carbon atoms in the pool of metabolized substrates. t_{50} equals the time at which 50% of carbon atoms were exchanged in the animals' breath.

hematophagous *Desmodus rotundus* or insectivorous *Myotis myotis* (Voigt et al., 2008a; Voigt et al., 2008c). Given that *R. aegyptiacus* is more than 10 times heavier than previously studied bats, it appears that paracellular transport of sugars facilitates the swift recruitment of dietary sugars for metabolism. It is important to note that the so-called incorporation rates of exogenous substrates into the pool of metabolized substrates includes four process: (1) ingestion and intestinal uptake of food, (2) oxidation of exogenous substrates, (3) equilibration of CO_2 labeled with the dietary isotopic signature in various bicarbonate pools, and (4) washout of CO_2 from the bicarbonate pool in exhaled breath. Time delays may occur during all four processes, and based on our data we are not able to determine the timeline of each dynamic action separately. Equilibration of newly oxidized CO_2 in low flux bicarbonate pools, such as in bone, may cause the longest delay, particularly in resting mammals with a low metabolic rate (Pallikarakis et al., 1991; Elia et al., 1992). However, if exchange of CO_2 among high flux and low flux bicarbonate pools delays the equilibration process, we can conservatively infer that the process of substrate oxidation may even be faster than the measured t_{50} values.

Metabolic substrate use in flying *R. aegyptiacus*

Previous respirometric measurements in *R. aegyptiacus* flying in a wind tunnel indicated that, in flight, bats catabolized mostly carbohydrates (Carpenter, 1986) but, until now, it was unclear whether these originated from endogenous (glycogen) or exogenous sources (dietary sugars). The results from our flight experiments indicate that, in general, the dynamics of metabolic substrate use is similar in resting and flying *R. aegyptiacus*. After ingestion of C4 sucrose, $\delta^{13}C$ of active bats reached –12.4±1.6‰, which is close to the –12‰ of ingested sugar. This result supports the hypothesis that the considerably increased energy demands of flight over rest are fuelled rapidly and probably exclusively by exogenous sugars. Potential delays caused by newly oxidized CO_2 getting trapped in low flux bicarbonate pools, such as bone, are probably negligible in active bats, since exchange rates among bicarbonate pools are enhanced during exercise (Pallikarakis et al., 1991).

We found that *R. aegyptiacus* fuel both flight and rest during nocturnal activity exclusively by catabolism of ingested sugars after being fed a sugar meal. There are several adaptive advantages to immediate and exclusive combustion of dietary sugars: (1) to reduce m_b, and thereby weight, flying animals should not store large energy reserves of glycogen and lipids (Caviedes-Vidal et al., 2007), (2) direct combustion of food circumvents the energy loss due to conversion of non-lipid precursor molecules into fat (Schmidt-Nielsen, 1997), and (3) saving fat during the normal daytime routine may help bats survive periods of adverse weather conditions such as cold temperatures or rain when food availability is limited (Korine et al., 1999a).

We also found that direct and rapid combustion of recently ingested food is not restricted to small bats and birds that feed on carbohydrate-rich diets (Voigt and Speakman, 2007; Voigt and Speakman, 2008a; Voigt and Speakman, 2008b; Welch et al., 2008), or on protein-rich diets (Voigt et al., 2008c; Voigt et al., 2010), but also occurs in larger flying vertebrates such as ~150 g *R. aegyptiacus*. Old World flying foxes (Pteropodidae) depend predominantly on sugar-rich food, with most species feeding on fruits and a minority on nectar, or a combination of the two (Marshall, 1985; Bhat, 1994; Korine et al., 1999a; Banack, 1998; Singaravelan and Marimuthu, 2004; Elangovan et al., 1999; Stier and Mildenstein, 2005). Many Pteropodids, particularly larger species, are known to cover long distances in their search for food [e.g. *Eidolon helvum* (Richter and Cumming, 2006); *Pteropus vampyrus* (Epstein et al., 2009); *R. aegyptiacus* (A. Tsoar, personal communication)]. During these commuting flights, the bats likely use flapping flight most of the time, although gliding has been observed in some species, e.g. *R. aegyptiacus* in wind tunnel experiments (Pennycuick, 1971). Our data suggest that flying pteropodid bats use ingested sugar directly and rapidly to fuel foraging flights between fruiting trees. Although the major advantage of the direct combustion of food sources might lie in the reduction of transport costs associated with surplus body reserves, some body lipids and also glycogen are obviously needed because pteropodid bats fly to their feeding sites around sunset after having fasted often for more than 12 h.

In summary, our data highlight the importance of direct combustion of exogenous substrates as a fuel for even comparatively large airborne mammals such as *R. aegyptiacus*. We conclude that exogenous fuels are probably important for all flying vertebrates with a carbohydrate-dominated diet.

LIST OF ABBREVIATIONS

C3	C3 photosynthetic pathway of CO_2 fixation in plants
C4	C4 photosynthetic pathway of CO_2 fixation in plants
IRMS	isotope ratio mass spectrometer
IR-SIA	infra-red stable isotope analyzer
k	fractional turnaround time of carbon atoms in the pool of metabolized substrates (min^{-1})
m_b	body mass (g)
MR	metabolic rate
t_{50}	time at which 50% of carbon atoms are exchanged in the animals' breath (min)
$\delta^{13}C$	stable carbon isotope ratio (‰)
$\delta^{13}C_{breath}$	stable carbon isotope ratio in exhaled breath (‰)
$\delta^{13}C_{breath}(\infty)$	stable carbon isotope ratio at time infinity (‰)
$\delta^{13}C_{breath}(0)$	stable carbon isotope ratio at time zero (‰)
$\delta^{13}C_{breath}(t)$	stable carbon isotope ratio at time t (‰)

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