Oxidation of dietary sugar during hovering flight in small vertebrate nectarivores

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There was an error published in Eqn 3 (p. 2156) of *J. Exp. Biol.* **210**, 2154-2162 and in Eqn 2 (p. 311) of *J. Exp. Biol.* **211**, 310-316.

In both papers, the following equation was written incorrectly as:

$$\delta^{13}C_{breath} = [\delta^{13}C_{ambient(fa)} + \delta^{13}C_{sample}] / 1 - f_a$$

The equation should have read:

$$\delta^{13}C_{breath} = [\delta^{13}C_{sample} - \delta^{13}C_{ambient(fa)}] / 1 - f_a$$

The authors apologise for any inconvenience this may have caused but assure readers that the error was typographical and does not affect the data, results, interpretations or conclusions of either paper.

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Dietary sugar as a direct fuel for flight in the nectarivorous bat *Glossophaga soricina*

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SUMMARY

It is thought that the capacity of mammals to directly supply the energetic needs of exercising muscles using recently ingested fuels is limited. Humans, for example, can only fuel about 30%, at most, of exercise metabolism with dietary sugar. Using indirect calorimetry, i.e. measurement of rates of O_2 consumption and CO_2 production, in combination with carbon stable isotope techniques, we found that nectarivorous bats *Glossophaga soricina* use recently ingested sugars to provide ~78% of the fuel required for oxidative metabolism during their energetically expensive hovering flight. Among vertebrate animals, only hummingbirds exceed the capacity of these nectarivorous bats to fuel exercise with dietary sucrose. Similar experiments performed on Anna's (*Calypte anna*) and rufous (*Selasphorus rufus*) hummingbirds show that they use recently ingested sugars to support ~95% of hovering metabolism. These results support the suggestion that convergent evolution of physiological and biochemical traits has occurred among hovering nectarivorous animals, rendering them capable of a process analogous to aerial refueling in aircraft.

Key words: bat, carbohydrate, energetics, fatty acid, stable isotope.

INTRODUCTION

In evolving to be small and to hover while feeding on floral nectar, neotropical Glossophagine bats have undergone evolutionary convergence with hummingbirds. Hovering flight is energetically costly; hovering hummingbirds achieve some of the highest known mass-specific metabolic rates among vertebrates (Suarez, 1992). During hovering, >90% of whole-body metabolic rate (rate of oxygen consumption, $\dot{V}_{O_2}$) is due to exercising flight muscles (Suarez, 1992; Taylor, 1987). Hover-feeding hummingbirds are almost exclusively able to fuel their highly aerobic flight muscles using recently ingested sugar (Welch et al., 2006; Welch and Suarez, 2007). In contrast, at moderate to high exercise intensities, humans and other mammals rely heavily on endogenous fuels (glycogen and triglyceride) stored in their locomotory muscles. This is thought to be due to their limited capacities to transport and metabolize exogenous fuels (glucose and fatty acids) from the blood (Weber et al., 1996; Weber, 1988). In addition, capacities for the assimilation, transport and oxidation of dietary fuels are also limited; in humans only about 25–30% of energy expenditure can be supported by sugar ingested shortly before (or during) exercise. Given their nectarivorous diet and phylogenetic status, it is of interest to determine the extent to which nectarivorous bats rely on recently ingested sugar to fuel their energetically expensive flight. Their small size, high wing-beat frequencies, high metabolic rates and reliance upon simple sugars to supply most of their daily energy needs (Norberg et al., 1993; Voigt and Speakman, 2007; Winter and von Helversen, 1998; Winter and von Helversen, 2001), led us to hypothesize that, like hummingbirds, they are able to directly fuel their exercising muscles using recently ingested sugar. To test this hypothesis, we used a combination of respirometric and stable carbon isotope techniques to compare the use of recently ingested sugar by nectarivorous bats and hummingbirds. The evidence obtained lends further support for the idea that physiological and biochemical traits have undergone evolutionary convergence among these nectarivorous, flying vertebrates.

MATERIALS AND METHODS

We evaluated the ability of Pallas' long-tongued nectar bats (*Glossophaga soricina* Pallas 1766) to support hovering metabolism with newly ingested sugar using a diet-switching approach. Bats were maintained for several weeks on a diet of beet sugar and powdered milk from cows fed a C3 crop. Hummingbirds were maintained for several weeks to months on a commercial hummingbird diet supplemented with beet sugar. The carbon in each of these diets displayed a stable isotope ratio ($^{13}C/^{12}C$) characteristic of C3 photosynthetic plants. During the experimental period, bats and hummingbirds were allowed to feed on a solution of cane sugar, the product of C4 photosynthesis with a significantly different ratio of $^{13}C/^{12}C$. Sugar solutions were dispensed by a tube inside a plastic mask into which ambient air was drawn and analyzed downstream for O_2 and CO_2 content. In addition to the estimation of relative rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) during periods when the bats and hummingbirds breathed through the mask, expired air was captured to determine the ratio of $^{13}C/^{12}C$ present in the CO_2 in it. Because of the difference in carbon stable isotopic signatures of beet and cane sugars and because the expired CO_2 is derived from the carbon of oxidized fuels, we were able to determine the source (i.e. endogenous C3 or dietary C4) of oxidized fuels and the time course over which the fuel source changed as the bats and hummingbirds continued to feed (Carleton et al., 2006; Welch et al., 2006).

We report $\delta^{13}C$ on a per mil (‰) basis relative to the international carbon standard, Vienna Pee Dee Belemnite (VPDB), where:

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

All solid and gas samples were submitted to the University of California, Santa Barbara Marine Science Institute Analytical Lab for analysis of $^{13}C/^{12}C$ ratios by mass spectrometry. This facility utilizes a Roboprep-CN stable isotope ratio mass spectrometer (Europa Scientific, Crewe, UK) equipped with an autosampler for introduction of gas samples into the continuous flow combustion chamber.

All capture, housing and experimental protocols were approved by the University of California, Santa Barbara Institutional Animal Care and Use Committee (Protocols 672 and 722). All data, except where noted, are presented as means ± s.e.m.

Experimental protocol

Individual Pallas' long-tongued nectar bats (*Glossophaga soricina* Pallas 1766; body mass at start of experiment=9.9±0.1 g; N=7, i.e. 3 males, 4 females) were captured *via* mist nets in banana plantations located near Tecomán, Colima, Mexico. Bats were transported to the city of Colima and were housed, indoors, in a wire-mesh cage of dimensions 0.5×0.5×0.5 m. Bats were fed *ad libitum* on a 20% (w/v) beet sugar solution supplemented with 5% (w/v) powdered cow's milk (Nestle Nido, Glendale, CA, USA) and 0.01% (w/v) ascorbic acid. The $\delta^{13}C$ value of this maintenance diet was –25.70±0.12‰ (VPDB, mean ± s.d., N=10).

Data collection on bats was conducted in a mesh tent approximately 2 m long×2 m wide×1 m high. Average temperature during all experiments was 21.6±0.1°C (range= 20.7–23.8°C). Bats were weighed while inside a cloth bag on an electronic balance immediately before and after participation in the experiment. Data collection took place during February and March 2007 between 23:00 h and 06:00 h. Bats were fasted during the day and prior to data collection. This simulated their natural cycle of feeding and fasting, ensured that they were motivated to feed, and maximized their reliance on fatty acid oxidation.

Night-time experiments commenced as bats were provided with a sugar cane sucrose solution (20% w/v). The $\delta^{13}C$ value of this solution was –11.22±0.12‰ (VPDB, mean ± s.d., N=10). Bats could access the sucrose solution by hovering in front of and inserting their head inside a plastic tube (derived from a 30 ml syringe) functioning as a mask. The solution was contained in a 30 ml syringe placed in a syringe pump (NE-500, New Era Pump Systems, Inc., Wantagh, NY, USA) and delivered to the mask by thin plastic tubing. An infrared emitter and detector were placed on opposite sides of the front edge of the mask such that the IR beam would be occluded whenever the bat's head was in the mask. Occlusion of IR beam triggered the release of sucrose solution by the syringe pump at a rate of 3 ml min^{-1}. This delivery rate was chosen because it resulted in the bats remaining in the mask for the longest period of time. The period of occlusion of the IR beam also represented the duration of the feeding event; this was the time over which measurement of reduction of $[O_2]$ and enhancement of $[CO_2]$ during hover-feeding occurred (see below). Air was continuously drawn into the mask at a rate of 500 ml min^{-1} by a pump and passed through a column of Drierite™ (W. A. Hammond Drierite, Xenia, OH, USA) to scrub it of water vapor before entering the CO_2 analyzer (CA-2A, Sable Systems International, Las Vegas, NV, USA). After leaving the CO_2 analyzer, the air was drawn through a Drierite-Ascarite-Drierite column (Ascarite II, Arthur H. Thomas, Philadelphia, PA, USA) to scrub CO_2 and residual water from the line and into the oxygen analyzer (FOXBOX, Sable Systems International). A thermistor was placed near the mask to record ambient temperature. Output from the gas analyzers, infrared detector and thermistor were recorded by a notebook computer using Expedata (version 1.0.17, Sable Systems International).

Immediately before data collection, the oxygen analyzer was calibrated with well-mixed ambient air drawn through the mask in the absence of a bat. The carbon dioxide analyzer was calibrated with CO_2-free nitrogen gas (zero gas) and 0.5% CO_2 in nitrogen gas (Praxair, Danbury, CT, USA).

STP-corrected O_2 depletion and CO_2 enrichment associated with each feeding event were determined by first subtracting baseline values (determined as the linear extrapolation of points directly before and after the feeding event in question) and then converting baseline-corrected data to ml gas by application of standard equations (Withers, 1977). Determination of absolute rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide ($\dot{V}_{CO_2}$) production was not possible during this experiment because subsampling of incurrent air was attempted in each case (see below). However, as subsampling likely did not discriminate between oxygen and carbon dioxide, relative volumes (ml) of oxygen and carbon dioxide respired by the bat were determined. Relative gas exchange rates (for use in calculating RQ values) were obtained by integration of depletion or enrichment peaks over time (min).

Because subsampling of expired gas for stable isotope analysis prior to analysis precluded determination of absolute rates of O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$), separate measurements of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ during hover-feeding were performed on each individual at a flow rate of 1200 ml min^{-1} without taking expired breath subsamples.

Expired CO_2 was collected for stable isotope analysis while bats were hover-feeding at the respirometry mask by drawing air from the incurrent airline approximately halfway between the mask and the carbon dioxide analyzer using a 60 ml syringe (Welch et al., 2006). These samples contained both ambient and expired CO_2. To estimate $\delta^{13}C$ of respired breath ($\delta^{13}C_{breath}$) we used a two-part concentration-dependent mixing model adapted from Phillips and Koch (Phillips and Koch, 2002), such that:

$$\delta^{13}C_{breath} = \frac{\delta^{13}C_{ambient}(f_a) + \delta^{13}C_{sample}}{1 - f_a} , \quad (2)$$

where $\delta^{13}C_{sample}$ is $\delta^{13}C$ of air collected in the syringe. $\delta^{13}C_{ambient}$ is the average $\delta^{13}C$ of air collected at four points during the 2 h experimental period (one within first 10 min, one at approximately the 30 min mark, one at approximately the 60 min mark, and one within 15 min of the end of the 2 h period) in the same manner as above when a bat was not present at the mask. f_a is the fraction of CO_2 in the gas sample from ambient air. Ambient $[CO_2]$ (p.p.m.) was determined using the CO_2 analyzer immediately before a feeding bout. $[CO_2]$ (p.p.m.) of the air sample was determined during stable isotope analysis *via* mass spectrometry. Immediately following collection, the contents of the 60 ml syringe were injected into pre-evacuated 12 ml Exetainer vials (Labco Ltd, Buckinghamshire, UK) until a positive pressure was achieved. Samples were stored at room temperature for up to 14 days before submission for analysis.

Comparison with hummingbirds

We obtained data from Anna's hummingbirds (*Calypte anna* Lesson 1829; body mass at start of experiment=4.4±0.4 g; *N*=2, both male) and rufous hummingbirds (*Selasphorus rufus* Gmelin 1788; body mass at start of experiment=3.2±0.1 g, *N*=10, i.e. 6 males, 4 females) for comparison with bats. Capture and rearing were essentially as described previously (Welch et al., 2007; Welch and Suarez, 2007). Birds were fed *ad libitum* on a 13% (w/v) solution of Nektar-Plus (Guenter Enderle, Tarpon Springs, FL, USA) supplemented with beet sugar (5% w/v). The $\delta^{13}C$ value of the maintenance diet was –25.84±0.11‰ (VPDB, mean ± s.d., *N*=10). Data collection took place between December 2005 and March 2006 between 06:00 h and 11:00 h in the laboratory at 24.0±0.1°C. Prior to each experiment, the hummingbird was fasted overnight to ensure that it would oxidize primarily fat at the start of data collection (Suarez et al., 1990; Welch et al., 2006). The experimental protocol was identical to that described for bats except that the duration was 60 min and the sucrose solution was contained in a 20 ml syringe placed directly in the mask. The $\delta^{13}C$ value of the sucrose solution used in this experiment was –11.69±0.11‰ (VPDB, mean ± s.d., *N*=10). Data for which f_a was greater than 0.5 were excluded as estimates of $\delta^{13}C_{sample}$ were less robust in these cases.

RESULTS

Respiratory quotients

Our captive bats typically commenced feeding after midnight. During the first hover-feeding bout following their daytime and evening fast, RQ ($=\dot{V}_{CO_2}/\dot{V}_{O_2}$) values, estimated by flow-through respirometry, were low (0.78±0.00; *N*=7), indicating a strong reliance on fatty acid metabolism to fuel flight. We used these data and the equations of Péronnet and Massicotte (Péronnet and Massicotte, 1991) to estimate rates of fat and carbohydrate oxidation in exercising bats, under the assumption that the contribution of protein catabolism to exercise metabolism is negligible (Bulow, 1988; Gessaman and Nagy, 1988; Vaillancourt et al., 2005). There are no published data concerning the contribution of protein catabolism to exercise metabolism in *G. soricina*; however, Herrera et al. (Herrera et al., 2006) measured low rates of nitrogen excretion in *G. soricina* and determined that their apparent maintenance nitrogen requirement was approximately 60 mg $kg^{-0.75}$ day^{-1}, much lower than most mammals and comparable to other nectarivorous animals. Thus, it appears unlikely that protein oxidation contributes significantly to the fuelling of metabolism in these bats. Using the caloric content of a unit mass of each substrate (Jeukendrup and Wallis, 2005), it is possible to determine the total rate of caloric expenditure during hovering flight (Met_{total}, cal min^{-1}; 1 cal=4.1868 J) and the rate of caloric expenditure resulting from oxidation of endogenous fat (Met_{fat}, cal min^{-1}). The percentage of metabolism fuelled by oxidation of endogenous fat (f_{fat}) is:

$$f_{fat} = 100 \left(\frac{Met_{fat}}{Met_{total}} \right). \quad (3)$$

f_{fat} averaged 74.0±1.5% (*N*=7) during the first feeding bout following the daily fasting period.

Bat RQ values quickly rose as foraging continued, approaching 1.0 (0.97±0.01; *N*=7) by about 30 min after feeding bouts commenced (Fig. 1A); this indicates that bats had switched to oxidizing predominantly carbohydrates. Because we relied on spontaneous, voluntary feeding behavior, variation among individuals in feeding frequencies (among both bats and hummingbirds) caused variation in the amount of time it took for RQ values to stabilize at or near 1.0, as well as in the amount of time required for $\delta^{13}C$ values in expired breath to stabilize near the $\delta^{13}C$ value of the experimental diet (below). In general, both RQ and $\delta^{13}C$ had stabilized to new steady-state values at least 30 min after foraging bouts had commenced.

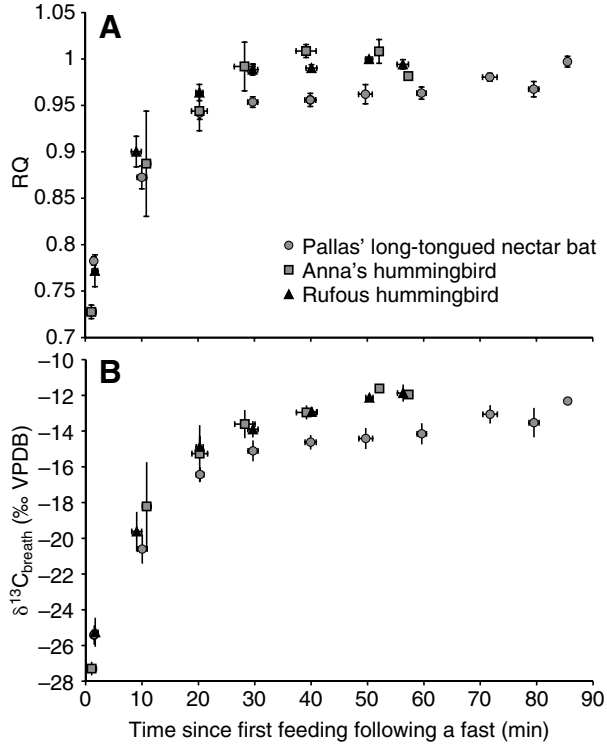

Fig. 1. Respiratory quotient ($RQ=\dot{V}_{CO_2}/\dot{V}_{O_2}$) (A) and $\delta^{13}C$ value of expired breath ($\delta^{13}C_{breath}$, ‰ VPDB) (B) *versus* time since first feeding following a fasting period (in min) for Anna's hummingbirds (*C. anna*; *N*=2) and rufous hummingbirds (*S. rufus*; *N*=10) as well as Pallas' long-tongued nectar bat (*G. soricina*; *N*=7). Data are averaged for each species.

Similar to results reported previously for hummingbirds (Welch et al., 2006; Welch and Suarez, 2007), RQ values for Anna's and rufous hummingbirds were initially low during the first feeding following a fasting period. RQ averaged 0.72±0.00 (*N*=2) for Anna's and 0.73±0.01 (*N*=5) for rufous hummingbirds during the first feeding following a fasting period. Calculated as above, using Eqn 3, f_{fat} values averaged 95.6±1.2% (*N*=2) for Anna's and 91.4±2.5% (*N*=5) for rufous hummingbirds during the first feeding following a fasting period, indicating the birds were oxidizing fatty acids almost exclusively to fuel their first foraging flight.

RQ values from hovering hummingbirds rose quickly with each successive foraging event, reaching average values near 1.0 (*C. anna*: 1.01±0.00; *N*=2; *S. rufus*: 0.99±0.00; *N*=10) by approximately 30 min after the first feeding following the fasting period (Fig. 1A), indicating essentially exclusive reliance on the oxidation of carbohydrates.

Stable isotopic signatures of expired breath

After being maintained on a diet with a $\delta^{13}C$ value of –25.70±0.12‰ (VPDB, mean ± s.d.), bats expired CO_2 that yielded correspondingly low average $\delta^{13}C_{breath}$ values during the first feeding following a fast (–25.71±0.56‰, VPDB; *N*=7) and were

not significantly different from the $\delta^{13}C$ signature of their maintenance diet (t_6=–0.0102, P=0.9920). This indicates reliance on endogenous fuel stores. Like RQ values, average $\delta^{13}C_{breath}$ values quickly rose as foraging continued (Fig. 1B), and approached (–14.11±0.64 ‰, VPDB; N=7) the $\delta^{13}C$ signature of the experimental sugar solution (–11.22‰, VPDB; t_6=–4.5307, P=0.0040). This indicates a significant shift towards reliance on exogenous sugar to fuel hovering flight.

The data from hummingbirds followed a similar pattern. During the first feeding following the fasting period, hummingbirds expired CO_2 with $\delta^{13}C_{breath}$ values that were not significantly different from their maintenance diet, which had a $\delta^{13}C$ value of –25.84±0.11‰ (VPDB, mean ± s.d.; *C. anna*: –27.78±0.49‰, VPDB, N=2, t_1=–4.3345, P=0.1443; *S. rufus*: –26.73±0.44‰, VPDB, N=5, t_4=–2.0234, P=0.1131), indicating reliance on endogenous energy stores. Like RQ values, species-averaged hummingbird $\delta^{13}C_{breath}$ values quickly rose as foraging continued (Fig. 1B), and approached (*C. anna*: –12.54±0.24‰, *VPDB*; N=2; *S. rufus*: –12.42±0.20‰, VPDB, N=10) the $\delta^{13}C$ signature of the experimental sugar solution. After 30 min of foraging, average $\delta^{13}C_{breath}$ values from Anna's hummingbirds were not significantly different from the experimental sucrose solution (–11.69‰, VPDB; t_1=–3.5087, P=0.1768). However, while average $\delta^{13}C_{breath}$ values from rufous hummingbirds during this period were close to the $\delta^{13}C$ signature of the experimental sugar solution, they were significantly lower (–11.69‰, VPDB; t_9=–3.5730, P=0.0060). As with the bats, this indicates a significant shift in hummingbirds towards reliance on exogenous sugar to fuel hovering flight.

To estimate the fractional rate of isotope incorporation into the pool of expired CO_2, a first-order negative exponential function was fitted to $\delta^{13}C_{breath}$ values during the experimental period for both bats and hummingbirds. We assume that the incorporation of carbon into expired CO_2 can be approximated by single-compartment, first-order kinetics (Carleton et al., 2006; Welch and Suarez, 2007). The non-linear fitting formula is:

$$\delta^{13}C_{breath}(t) = \delta^{13}C_{breath}(\infty) + [\delta^{13}C_{breath}(0) = \delta^{13}C_{breath}(\infty)]e^{-kt}, \quad (4)$$

where $\delta^{13}C_{breath}(t)$ is the isotope composition of the carbon in expired CO_2 at time t, $\delta^{13}C_{breath}(0)$ is the estimated initial isotope composition of the carbon in expired CO_2, $\delta^{13}C_{breath}(\infty)$ is the asymptotic equilibrium isotope composition of the carbon in expired CO_2 and k is the fractional rate of isotope incorporation into the pool of expired CO_2 (Carleton et al., 2006; Carleton and Martínez del Rio, 2005; O'Brien et al., 2000; Welch and Suarez, 2007).

The average percentage rate of isotope incorporation into the pool of expired CO_2 (k'= k×100) in bats was 7.0±1.7% per min (range: 3.4–9.5%, N=7). In Anna's hummingbirds the average percentage rate of isotope incorporation into the pool of expired CO_2 (k') was 7.8±3.2% min^{-1} (range: 3.3–12.2%, N=2). In rufous hummingbirds, the average value of k' was 11.8±1.4% min^{-1} (range: 4.0–24.1%, N=10).

$\delta^{13}C_{breath}$ *vs* RQ

RQ and $\delta^{13}C_{breath}$ values are highly significantly correlated for each of the three species examined here (data pooled by species; Fig. 2; *G. soricina*: r_{91}=0.9374, P<0.0001; *C. anna*: r_{22}=0.9767, P<0.0001; *S. rufus*: r_{81}=0.9185, P<0.0001). This indicates that newly ingested sugars were the primary source of the carbohydrates oxidized during hovering flight.

Oxidation rate of exogenous sugars

The oxidation rate of exogenous sugars (M_{exo}, mg min^{-1}) is calculated as:

$$M_{exo} = \frac{\dot{V}_{CO_2}\left(\frac{\delta^{13}C_{breath} - \delta^{13}C_{endo}}{\delta^{13}C_{exo} - \delta^{13}C_{endo}}\right)}{g}, \quad (5)$$

where $\dot{V}_{CO_2}$ is the whole-body CO_2 production rate (ml min^{-1}), $\delta^{13}C_{endo}$ is the average of $\delta^{13}C_{breath}$ values from the first feeding following a fast (resulting from oxidation of solely endogenous fuels), $\delta^{13}C_{exo}$ is the $\delta^{13}C$ of the exogenous fuel and g is the volume of CO_2 produced by glucose oxidation (g=0.7426 ml mg^{-1}) (Adopo et al., 1994).

Upon reaching steady state after feeding commenced (>30 min after the first feeding following a fast), M_{exo} averaged 3.80±0.22 mg min^{-1} (N=7, Table 1). The bats' mass-specific rate of oxidation of exogenous sugars (M_{exo}/M_b, in mg sugar min^{-1} g^{-1} body mass) averaged 0.37±0.02 mg min^{-1} g^{-1} (N=7) during the same period (Table 1). Upon reaching steady state after feeding commenced, whole animal M_{exo} values were generally similar in Anna's and rufous hummingbirds, averaging 3.49±0.08 and 2.45±0.07 mg min^{-1}, respectively. M_{exo}/M_b values averaged 0.72±0.05 mg min^{-1} (N=2) and 0.71±0.01 mg min^{-1} (N=10) during feeding bouts upon reaching steady state after feeding commenced in Anna's and rufous hummingbirds, respectively (Table 1).

DISCUSSION

The data presented here indicate that Pallas' long-tongued nectar bats oxidize recently ingested sugar during hovering (3.80±0.22 mg min^{-1}) at a rate very similar to hovering hummingbirds (*C. anna*, 3.49±0.08 mg min^{-1}; *S. rufus*, 2.45±0.07 mg min^{-1}). Correcting for differences in body mass, the bats achieved mass-specific oxidation rates of recently ingested sugar (0.37±0.02 mg min^{-1} g^{-1}) that are slightly more than half of the rates achieved by the hummingbirds (*C. anna*, 0.72±0.05 mg min^{-1} g^{-1}; *S. rufus*, 0.71±0.01 mg min^{-1} g^{-1}). In an

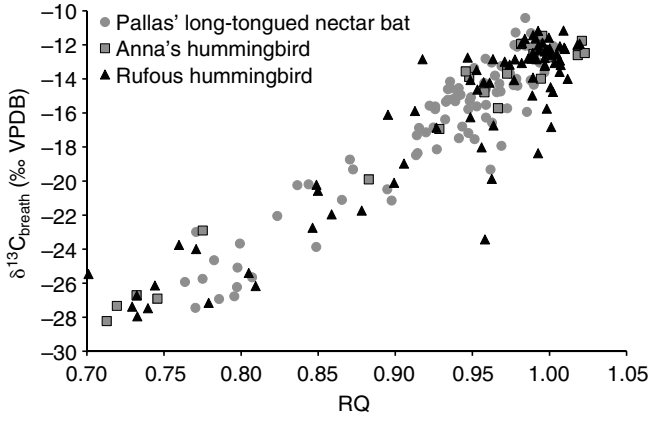

Fig. 2. Relationship between respiratory quotient (RQ=$\dot{V}_{CO_2}/\dot{V}_{O_2}$) and $\delta^{13}C$ value of expired breath ($\delta^{13}C_{breath}$, ‰ VPDB) during the same hover-feeding event in Anna's hummingbirds (*C. anna*; N=2) and rufous hummingbirds (*S. rufus*; N=10) as well as Pallas' long-tongued nectar bats (*G. soricina*; N=7). Data are pooled for each species. Pairwise comparisons reveal a significant correlation between these variables for each species (*C. anna*: r_{22}=0.9767, P<0.0001; *S. rufus*: r_{81}=0.9185, P<0.0001; *G. soricina*: r_{91}=0.9374, P<0.0001).

Table 1. Isotopic incorporation rate, percentage of metabolism supported by oxidation of exogenous sugar, total and mass-specific oxidation rate of exogenous sugar and mass-specific oxygen consumption rate of Pallas' long-tongued nectar bats *Glossophaga soricina*, and hummingbirds *Selasphorus rufus* and *Calypte anna* during hovering and humans (*Homo sapiens*) during exercise at 50% of maximal oxygen consumption rate*

Species	Average M_b (g)	k' (% min^{-1})	f_{exo}	M_{exo} (mg min^{-1})	M_{exo}/M_b (mg min^{-1} g^{-1})	M_{exo}/M_b (mg min^{-1} kg^{-1})	$\dot{V}_{O_2}/M_b$ (ml g^{-1} h^{-1})
Homo sapiens	74 100±1900 (9)	–	32.4	1220±70 (9)	0.02	16.5	2.31
Glossophaga soricina	10.2±0.1 (7)	7.0±1.7 (7)	77.6±4.8 (7)	3.80±0.22 (7)	0.37±0.02 (7)	366.8±20.8 (7)	21.29±0.60 (7)
Selasphorus rufus	3.4±0.1 (10)	11.8±1.4 (10)	94.7±1.5 (10)	2.45±0.07 (10)	0.71±0.01 (10)	713.7±13.4 (10)	33.70±0.45 (10)
Calypte anna	4.8±0.3 (2)	7.8±3.2 (2)	95.5±1.5 (2)	3.49±0.08 (2)	0.72±0.05 (2)	715.0±48.5 (2)	33.36±1.76 (2)

Values are means ± s.e.m. (*N*).
k', isotopic incorporation rate; f_{exo}, percentage of metabolism supported by oxidation of exogenous sugar; M_{exo}, total and M_{exo}/M_b, mass-specific oxidation rate of exogenous sugar; $\dot{V}_{O_2}/M_b$ mass-specific oxygen consumption rate; $\dot{V}_{O_2max}$, maximal oxygen consumption rate.
*(Jentjens et al., 2004b).

experimental design closely approximating that used in our studies on hummingbirds and bats, Jentjens et al. (Jentjens et al., 2004b) report maximal rates of oxidation of exogenous sugars of 1220±70 mg min^{-1} (*N*=9) in exercising humans during a period 120–150 min after the initial ingestion of a glucose + sucrose solution (Table 1). Their data yield an average mass-specific rate of oxidation of exogenous sugars of 0.02 mg min^{-1} g^{-1} (16.5 mg min^{-1} kg^{-1}; Table 1). The mass-specific rate of oxidation of exogenous sugar displayed by Pallas' long-tongued nectar bats is about 18 times greater than the rate estimated in humans and is the highest rate ever reported in mammals. These results add further support to the hypothesis of convergent evolution in physiological and biochemical traits among hovering nectarivorous animals. In addition, they indicate that the traditional paradigm, i.e. that exogenous, dietary fuel use is highly limited in mammals during high intensity exercise, is not without exception.

In addition to their high capacity for oxidation of exogenous sugars, bats appear to be similar to hummingbirds in being able to use exogenous sugars to fuel hovering metabolism soon after ingestion. The range of fractional rates of isotopic incorporation into the pool of expired CO_2 was large within each species. This is undoubtedly due to the variation in feeding frequency and ingestion rate observed across individuals. In addition, variation in the rapidity with which ingested sugar appears in the pool of actively metabolized substrates may be due to the transit-time of the ingested sugar solution from storage organs, such as the crop and stomach, to the small intestine, where most sugar absorption occurs. These were not measured in the present study. Nonetheless, rates of incorporation of exogenous sugar into the pool of actively metabolized substrates seen in bats and hummingbirds exceed the rates observed in humans (Jentjens et al., 2004b).

Multiplying the rate of exogenous sugar oxidation (M_{exo}) by the caloric content of a given mass of sugar (Jeukendrup and Wallis, 2005) yields the rate of caloric expenditure resulting from oxidation of exogenous substrate (Met_{exo}, cal min^{-1}). The percentage of metabolism fuelled by oxidation of exogenous sugar (f_{exo}) is:

$$f_{exo} = 100 \left(\frac{Met_{exo}}{Met_{total}} \right) . \quad (6)$$

f_{exo} averaged 77.6±4.8% (*N*=7) upon reaching steady state after feeding commenced in Pallas' long-tongued nectar bats (Fig. 3). The proportion of hovering metabolism supported by exogenous sucrose reported here is strikingly similar to the proportion of resting metabolism supported by dietary sucrose reported recently

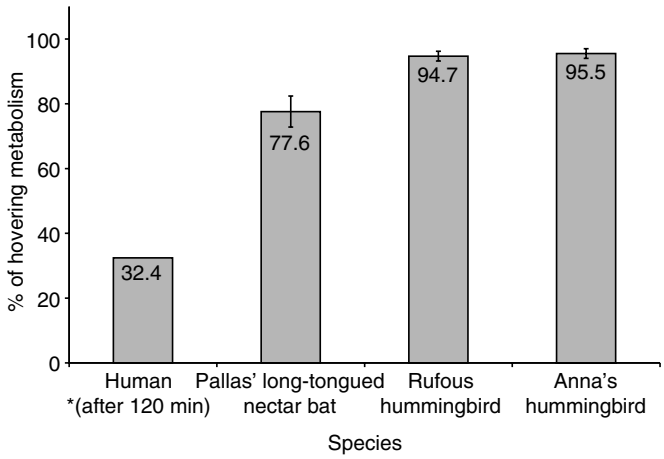

Fig. 3. Percentage of hovering (ergometer exercise in humans) metabolism supported by oxidation of exogenous sugar (f_{exo}) during a period beginning 30 min after initial sugar solution ingestion. Values are means ± s.e.m. *(Jentjens et al., 2004b).

(Voigt and Speakman, 2007). f_{exo} averaged 95.5±1.5% (*N*=2) and 94.7±1.5% (*N*=10) during the same period of time and at steady state in Anna's and rufous hummingbirds, respectively (Fig. 3). In humans, the average f_{exo} value during a period 120–150 min after initial ingestion of a glucose + sucrose solution is 32.4% (Fig. 3) (Jentjens et al., 2004b). These results support our hypothesis that Pallas' long-tongued nectar bats have, like hummingbirds, evolved capacities for fuelling hovering metabolism mainly using recently ingested sugar.

One factor limiting the use of exogenous sugars during exercise in humans is the capacity for carbohydrate absorption by the intestine (Hawley et al., 1992; Jentjens et al., 2004a). Rates of sugar absorption by hummingbird intestines are exceptionally high (Karasov et al., 1986; McWhorter et al., 2006) and a large fraction of the sugar absorption rate occurs *via* a paracellular pathway (McWhorter et al., 2006). Pallas' long-tongued nectar bats also possess the capacity for high rates of sugar absorption (Winter, 1998). Although data distinguishing between active transport and paracellular movement are currently not available for nectarivorous bats, studies on both the Egyptian fruit bats *Rousettus aegyptiacus* and the great fruit bat *Artibeus literatus* demonstrate that these also possess high capacities for sugar transport *via* a paracellular pathway (Caviedes-Vidal et al., 2004; Tracy et al., 2007). In hummingbirds, rates of active sugar transport in the intestines are insufficient and high rates of

paracellular transport are required to fully meet daily energy requirements (McWhorter et al., 2006). Given the high rates of daily energy expenditure and energetically expensive hovering flight of Pallas' long-tongued nectar bats, it is reasonable to expect that they would also rely heavily on a paracellular pathway for sugar absorption. Egyptian fruit bats experience much greater rates of paracellular absorption than similar-sized laboratory rats (Tracy et al., 2007). In non-volant mammals, the ratio of paracellular absorption to active transport of glucose increased with body mass, i.e. paracellular absorption made a minimal contribution in mice, the smallest mammals examined (Pappenheimer, 1990). This pattern supports the argument that it is not small size *per se* but, rather, convergent evolution among these flying nectarivores that resulted in their increased capacities for paracellular absorption of sugars.

Despite their remarkable abilities, it is interesting that the bats in our study failed to support hovering metabolism with exogenous sucrose to the same extent seen in hummingbirds. The RQ values obtained from bats foraging for at least 30 min were significantly lower than 1.0 (0.97±0.01, N=7; t_6=0.01711. P=0.0017). In contrast, RQ values obtaining from hummingbirds foraging for at least 30 min were 1.01±0.00 (N=2) in the case of *C. anna* and 0.99±0.00 (N=10) in *S. rufus*. This suggests that, even when nectar is available, bats continue to rely to some extent on fat oxidation to fuel hovering flight. Using Eqn 6, we calculate that bats foraging for at least 30 min support 10.3±2.1% (N=7, t_6=4.7915, P=0.0030) of hovering metabolism with fat. In comparison, Anna's and rufous hummingbirds support essentially none of their hovering metabolism with fat (i.e. not significantly different from zero; *C. anna*: –4.5±0.4%, N=2, t_1=–11.711, P=0.0542; *S. rufus*: 1.9±1.2%, N=10, t_9=1.5142, P=0.1643).

When we constrain calculated values for the percentage of hovering metabolism supported by exogenous carbohydrate and endogenous fat to between 0–100%, the balance of bat hovering metabolism not supported by either fat or exogenous carbohydrates was 12.1±2.9% (N=7). This likely represents the fraction of metabolism fuelled by endogenous glycogen, derived from carbon in the maintenance diet. In comparison, we calculate that Anna's and rufous hummingbirds fuel lower fractions of their hovering metabolism with endogenous carbohydrates (*C. anna*, 3.4±1.7%, N=2; *S. rufus*, 4.3±0.8%, N=10). The support of nearly 1/8th of hovering metabolism with endogenous glycogen differentiates bats from hummingbirds which, during hover-feeding, appear to rely on oxidation of glycogen previously synthesized from the maintenance diet to a lesser extent (Suarez et al., 1990; Welch et al., 2006; Welch and Suarez, 2007). During the first feeding following a fasting period, the bats relied on endogenous carbohydrates, likely in the form of glycogen, to fuel 27.7±3.7% of hovering metabolism. In comparison, Anna's and rufous hummingbirds supported only 8.1±1.0 and 7.1±4.0%, respectively, of hovering flight during the first feeding following a fasting period with endogenous carbohydrates. Thus, it appears that under both fasting and fed conditions, bats rely upon endogenous glycogen to fuel hovering flight to a much greater extent than hummingbirds.

A caveat to our interpretation of these data is that the floral nectars typically consumed by Phyllostomid nectarivorous bats and hummingbirds differ in the relative abundances of component sugars. On average, the sugars in 'bat nectars' consist of 20% sucrose, while the sugars in 'hummingbird nectars' consist of 60% sucrose (the balance consisting of the monosaccharides fructose and glucose) (Baker et al., 1998). Thus, their specialization on flowers producing low-sucrose nectars may explain why bats, despite their remarkable abilities, are unable to fuel their flight muscles with dietary sucrose to the same extent as hummingbirds. Consistent with this interpretation, Hernandez and Martínez del Rio determined that sucrase activities per unit surface area of intestine in Pallas' long-tongued nectar bats are approximately half those measured in hummingbirds (Hernandez and Martínez del Rio, 1992). Thus, it is possible that the oxidation rate of exogenous sugars by hovering bats is more constrained by limitations in the rate of sucrose hydrolysis (and subsequent absorption into the circulation) than in hovering hummingbirds. This suggests that nectarivorous bats may be capable of fueling 100% of flight metabolism when provided a mix of sucrose, glucose and fructose mimicking the nectar compositions they feed on in nature. In their recent study, Voigt and Speakman demonstrate that Pallas' long-tongued nectar bats are able to fuel a greater proportion of their resting metabolism with exogenous sugars when provided with glucose, as opposed to sucrose (Voigt and Speakman, 2007). However, extrapolation to foraging flight may not be appropriate because metabolic rates are much higher and due mainly to skeletal muscles under these conditions.

We expect that the results presented here will lead to further questions concerning the mechanistic bases for the exceptional abilities of bats to fuel exercise using recently ingested sugar, the evolution of these capacities, and their coevolution with bat-visited flowering plants. An increased understanding of the mechanisms underlying the high rates of dietary sugar metabolism among vertebrate endotherms may lead to insights into certain metabolic pathologies and their evolution in humans.

LIST OF SYMBOLS AND ABBREVIATIONS

$\delta^{13}C$	isotopic $^{13}C/^{12}C$ ratio referenced to international standard
f	fraction of expired CO_2 derived from metabolic substrate
g	volume CO_2 produced by glucose oxidation
M	amount of metabolic substrate oxidized (mg min^{-1})
M_b	body mass (g)
Met	rate of caloric expenditure (cal min^{-1})
RQ	respiratory quotient ($=\dot{V}_{CO_2}/\dot{V}_{CO_2}$)
t	time (min)
$\dot{V}_{CO_2}$	rate of carbon dioxide production (ml CO_2 g^{-1} h^{-1})
$\dot{V}_{O_2}$	rate of oxygen consumption (ml O_2 g^{-1} h^{-1})
VPDB	Vienna Pee Dee Belemnite C standard

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REFERENCES

Adopo, E., Péronnet, F., Massicotte, D., Brisson, G. R. and Hillaire-Marcel, C. (1994). Respective oxidation of exogenous glucose and fructose given in the same drink during exercise. *J. Appl. Physiol.* **76**, 1014-1019.

Baker, H. G., Baker, I. and Hodges, S. A. (1998). Sugar composition of nectars and fruits consumed by birds and bats in the tropics and subtropics. *Biotropica* **30**, 559-586.

Bulow, J. (1988). Lipid mobilization and utilization. In *Principles of Exercise Biochemistry* (ed. J. R. Poortmans), pp. 140-163. Basel: Karger.

Carleton, S. A. and Martínez del Rio, C. (2005). The effect of cold-induced increased metabolic rate on the rate of C-13 and N-15 incorporation in house sparrows (*Passer domesticus*). *Oecologia* **144**, 226-232.

Carleton, S. A., Bakken, B. H. and Martínez del Rio, C. (2006). Metabolic substrate use and the turnover of endogenous energy reserves in broad-tailed hummingbirds (*Selasphorus platycercus*). *J. Exp. Biol.* **209**, 2622-2627.

Caviedes-Vidal, E., Chadwick, J. G., Cruz-Neto, A. P. and Karasov, W. H. (2004). Sugar absorption in bats: are they mammals or birds? *Integr. Comp. Biol.* **44**, 534.

Gessaman, J. A. and Nagy, K. A. (1988). Energy-metabolism: errors in gas-exchange conversion factors. *Physiol. Zool.* **61**, 507-513.

Hawley, J. A., Dennis, S. C., Nowitz, A., Brouns, F. and Noakes, T. D. (1992). Exogenous carbohydrate oxidation from maltose and glucose ingested during prolonged exercise. *Eur. J. Appl. Physiol.* **64**, 523-527.

Hernandez, A. and Martínez del Rio, C. (1992). Intestinal disaccharides in five species of phyllostomid bats. *Comp. Biochem. Physiol.* **103B**, 105-111.
Herrera, L. G., Ramírez, N. and Mirón, L. (2006). Ammonia excretion increased and urea excretion decreased in urine of a new world nectarivorous bat with decreased nitrogen intake. *Physiol. Biochem. Zool.* **79**, 801-809.
Jentjens, R. L. P. G., Moseley, L., Waring, R. H., Harding, L. K. and Jeukendrup, A. E. (2004a). Oxidation of combined ingestion of glucose and fructose during exercise. *J. Appl. Physiol.* **96**, 1277-1284.
Jentjens, R. L. P. G., Venables, M. C. and Jeukendrup, A. E. (2004b). Oxidation of exogenous glucose, sucrose, and maltose during prolonged cycling exercise. *J. Appl. Physiol.* **96**, 1285-1291.
Jeukendrup, A. E. and Wallis, G. A. (2005). Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int. J. Sports Med.* **26**, S28-S37.
Karasov, W. H., Phan, D., Diamond, J. M. and Carpenter, F. L. (1986). Food passage and intestinal nutrient absorption in hummingbirds. *Auk* **103**, 453-464.
McWhorter, T. J., Bakken, B. H., Karasov, W. H. and Martínez del Rio, C. (2006). Hummingbirds rely on both paracellular and carrier-mediated intestinal glucose absorption to fuel high metabolism. *Biol. Lett.* **2**, 131-134.
Norberg, U., Kunz, T., Steffensen, J., Winter, Y. and von Helversen, O. (1993). The cost of hovering and forward flight in a nectar-feeding bat, *Glossophaga soricina*, estimated from aerodynamic theory. *J. Exp. Biol.* **182**, 207-227.
O'Brien, D. M., Schrag, D. P. and Martínez del Rio, C. (2000). Allocation to reproduction in a hawkmoth: a quantitative analysis using stable carbon isotopes. *Ecology* **81**, 2822-2831.
Pappenheimer, J. R. (1990). Paracellular intestinal absorption of glucose, creatinine, and mannitol in normal animals: relation to body size. *Am. J. Physiol.* **259**, G290-G299.
Péronnet, F. (2003). Oxydation des glucides ingeres pendant l'exercice. *Actual. Chim.* **8-9**, 34-37.
Péronnet, F. and Massicotte, D. (1991). Table of nonprotein respiratory quotient: an update. *Can. J. Sport Sci.* **16**, 23-29.
Phillips, D. L. and Koch, P. L. (2002). Incorporating concentration dependence in stable isotope mixing models. *Oecologia* **130**, 114-125.
Suarez, R. K. (1992). Hummingbird flight: sustaining the highest mass-specific metabolic rates among vertebrates. *Experentia* **48**, 565-570.
Suarez, R. K., Lighton, J. R. B., Moyes, C. D., Brown, G. S., Gass, C. L. and Hockachka, P. W. (1990). Fuel selection in rufous hummingbirds: ecological implications of metabolic biochemistry. *Proc. Natl. Acad. Sci. USA* **87**, 9207-9210.
Taylor, C. R. (1987). Structural and functional limits to oxidative metabolism: insights from scaling. *Annu. Rev. Physiol.* **49**, 135-146.
Tracy, C. R., McWhorter, T. J., Korine, C., Wojciechowski, M. S., Pinshow, B. and Karasov, W. H. (2007). Absorption of sugars in the Egyptian fruit bat (*Rousettus aegyptiacus*): a paradox explained. *J. Exp. Biol.* **210**, 1726-1734.
Vaillancourt, E., Prud'Homme, S., Haman, F., Guglielmo, C. G. and Weber, J.-M. (2005). Energetics of a long-distance migrant shorebird (*Philomachus pugnax*) during cold exposure and running. *J. Exp. Biol.* **208**, 317-325.
Voigt, C. and Speakman, J. (2007). Nectar-feeding bats fuel their high metabolism directly with exogenous carbohydrates. *Funct. Ecol.* **21**, 913-921.
Weber, J. M. (1988). Design of exogenous fuel supply-systems: adaptive strategies for endurance locomotion. *Can. J. Zool.* **66**, 1116-1121.
Weber, J., Roberts, T., Vock, R., Weibel, E. and Taylor, C. (1996). Design of the oxygen and substrate pathways. III. Partitioning energy provision from carbohydrates. *J. Exp. Biol.* **199**, 1659-1666.
Welch, K. C., Jr and Suarez, R. K. (2007). Oxidation rate and turnover of ingested sugar in hovering Anna's (*Calypte anna*) and rufous (*Selasphorus rufus*) hummingbirds. *J. Exp. Biol.* **210**, 2154-2162.
Welch, K. C., Bakken, B. H., Martínez del Rio, C. and Suarez, R. K. (2006). Hummingbirds fuel hovering flight with newly ingested sugar. *Physiol. Biochem. Zool.* **79**, 1082-1087.
Welch, K. C., Jr, Altshuler, D. L. and Suarez, R. K. (2007). Oxygen consumption rates in hovering hummingbirds reflect substrate-dependent differences in P/O ratios: carbohydrate as a 'premium fuel'. *J. Exp. Biol.* **210**, 2146-2153.
Winter, Y. (1998). *In vivo* measurement of near maximal rates of nutrient absorption in a mammal. *Comp. Biochem. Physiol.* **119A**, 853-859.
Winter, Y. and von Helversen, O. (1998). The energy cost of flight: do small bats fly more cheaply than birds? *J. Comp. Physiol. B* **168**, 105-111.
Winter, Y. and von Helversen, O. (2001). Bats as pollinators: foraging energetics and floral adaptations. In *Cognitive Ecology of Pollination* (ed. L. Chittka and J. Thomson), pp. 148-170. Cambridge: Cambridge University Press.
Withers, P. C. (1977). Measurement of V_{O_2}, V_{CO_2}, and evaporative water loss with a flow-through mask. *J. Appl. Physiol.* **42**, 120-123.