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Flight muscle enzymes and metabolic flux rates during hovering flight of the nectar bat, *Glossophaga soricina*: Further evidence of convergence with hummingbirds

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ABSTRACT

Given their high metabolic rates, nectarivorous diet, and ability to directly fuel their energetically-expensive flight using recently-ingested sugar, we tested the hypothesis that Pallas long tongued nectar bats (*Glossophaga soricina*) possess flight muscles similar to those of hummingbirds with respect to enzymatic flux capacities in bioenergetic pathways. In addition, we compared these biochemical capacities with flux rates achieved *in vivo* during hovering flight. Rates of oxygen consumption ($\dot{V}_{O_2}$) were measured during hover-feeding and used to estimate rates of ATP turnover, glucose and long-chain fatty acid oxidation per unit mass of flight muscle. Enzyme V_{max} values at key steps in glucose and fatty acid oxidation obtained *in vitro* from pectoralis muscle samples exceed those found in the locomotory muscles of other species of small mammals and resemble data obtained from hummingbird flight muscles. The ability of nectar bats and hummingbirds to hover in fed and fasted states, fueled almost exclusively by carbohydrate or fat, respectively, allowed the estimation of fractional velocities (v/V_{max}) at both the hexokinase and carnitine palmitoyltransferase-2 steps in glucose and fatty acid oxidation, respectively. The results further support the hypothesis of convergent evolution in biochemical and physiological traits in nectar bats and hummingbirds.

1. Introduction

Nectarivorous bats have converged with hummingbirds in evolving to be small and in feeding on floral nectar. Pallas long tongued nectar bats (*Glossophaga soricina*, referred to, henceforth, as "nectar bats"), in particular, are able to engage in energetically expensive hovering flight for up to several seconds while feeding (Voigt and Winter, 1999; Winter et al., 1998; Welch et al., 2008). Recent studies revealed that nectar bats are able to make use of recently-ingested sugars to directly fuel their metabolism (Voigt and Speakman, 2007). This is made possible, at least in part, by their high intestinal capacities for sugar assimilation (Hernández and Martínez del Rio, 1992; Winter, 1998), a physiological trait shared with hummingbirds (McWhorter et al., 2006). Previously, we performed experiments to determine whether nectar bats could, like hummingbirds (Welch et al., 2006, 2007), fuel hover-feeding using recently-ingested sugar. These studies yielded further evidence of their evolutionary convergence with hummingbirds: close to 80% of the energy required for hovering flight in nectar bats is provided by the oxidation of recently-ingested sugar (Welch et al., 2008). This is a remarkable feat for a mammal, given that humans can fuel only about 30% of exercise metabolism (Jentjens et al., 2004), while hummingbirds (*Selasphorus rufus* and *Calypte ana*) can fuel virtually all of their hovering metabolism directly using recently ingested sugar (Welch et al., 2007).

We measured the maximum capacities for flux, i.e, V_{max} values, *in vitro* at key steps in muscle energy metabolism to gain insights into the biochemical bases for the high rates of sugar oxidation estimated *in vivo* in hovering nectar bats and to further probe the extent of their convergence with hummingbirds. In addition, rates of glucose and fatty acid oxidation in the flight muscles, estimated from respirometry data, are compared with enzyme V_{max} values estimated *in vitro* to gain insights into the relationships between biochemical flux capacities and physiological flux rates during flight.

2. Materials and methods

2.1. Respirometry

Pallas long tongued nectar bats, *Glossophaga soricina*, were caught using mist nets in banana plantations near Colima, Mexico, and reared as described previously (Welch et al., 2008). Hover-feeding experiments were conducted in a large, well-ventilated camping tent in

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which the bats flew and fed at will. Measurements of O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) rates during hovering were performed by inducing bats to feed on sugar solutions from a mask modified to function as part of a flow-through respirometry system (Sable Systems, Las Vegas, NV, USA) that drew air into the mask at a flow rate of 1200 mL min^{-1}. The mask was fitted with an infra-red sensor that signaled when a bat's head was in the mask. Sucrose (cane sugar) solution (20% w/v) was dispensed within the mask at a rate of 3 mL min^{-1} by a syringe pump (NE-500, New Era Pump Systems, Wantagh, NY, USA) that was activated by the presence of the bat's head. The duration of this infrared signal allowed measurement of the duration the bat was respiring in the mask. The O_2 analyzer (FOX respirometer, Sable Systems) was calibrated using well-mixed ambient air; the CO_2 analyzer (CA-2A, Sable Systems) was calibrated using CO_2-free Nitrogen and 0.5% CO_2 in Nitrogen (Praxair, Danbury, CT, USA). Experiments were conducted in February and March 2007 between 23:00 and 06:00 h when ambient temperature (measured proximal to the mask) ranged from 20.7 to 23.8 °C. Bats were weighed using a portable, digital balance accurate to ± 0.1 g. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ values for each individual were calculated using Expedata software (version 1.0.17, Sable Systems).

All capture, handling, rearing and experimental protocols were approved by the University of California, Santa Barbara, Animal Care and Use Committee.

2.2. Sample preparation

The bats used for respirometry measurements were euthanized for enzyme measurements by cervical dislocation. Pectoral muscles were quickly dissected out, placed in screw-top cryovials, frozen in dry ice, and stored at −70 °C. Samples were kept in dry ice during transport to Santa Barbara for analysis and kept at −70 °C.

Improvements in tissue extraction procedures involving the use of the detergent, Triton X-100, and sonication of tissue homogenates (as described in Suarez et al., 1990) have led to large increases in the maximal enzyme activities originally reported by Suarez et al. (1986) in hummingbirds. We used these improved procedures in the present study on bats and, for comparison, performed new measurements using frozen pectoralis muscles from rufous hummingbirds (*Selasphorus rufus*). Hummingbirds were euthanized and muscle samples were dissected and frozen as described above. About 50 mg of bat or hummingbird muscle was cut from each frozen sample at a time, weighed, minced and homogenized in 9 volumes of one of the following buffers (indicated pH values are at room temperature): 25 mM HEPES (pH 7.0), 50 mM imidazole (pH 7.1), 50 mM Tris–Cl (pH 7.2) or sodium phosphate (pH 7.4) containing 2 mM EDTA, 0.5% Triton X-100 and 5 mM β-mercaptoethanol, except in the case of assays requiring 5,5′-dithio(2-nitrobenzoic acid) (DTNB, see below). Homogenization in ice-cold buffer was conducted for 10 s, 3 times at medium speed using a Pro 200 homogenizer (Pro Science, Oxford, CT, USA) fitted with the smallest available probe. Homogenates were sonicated for 10 s, three times using a Microson Ultrasonic Cell Disruptor model # MS-50 (Heat Systems Ultrasonics Inc., Farmingdale, NY, USA) at about 1/3 of maximum output while keeping samples ice-cold, then centrifuged for 4 min at 10,000 ×*g* at 4 °C using an IEC Micromax refrigerated microcentrifuge (Needham Heights, MA, USA). Supernatant fractions in microcentrifuge tubes were kept in crushed ice until assays were completed.

2.3. Enzyme assays

Assays were conducted essentially as described by (Suarez et al., 1986) in 1 mL glass cuvettes using a Shimadzu UV-160U recording spectrophotometer equipped with a water-jacketed cuvette holder. Assay temperature was regulated at 37 °C using a Grant circulating water bath (model LTD 6, Cambridge, UK). Assay conditions and controls for the measurement of V_{max} values for glycogen phosphorylase (GP, EC 2.4.1.1), hexokinase (HK; EC 2.7.1.1), phosphofructokinase (PFK; EC 2.7.1.11), carnitine palmitoyltransferase (CPT; EC 2.3.1.21), 3-hydroxyacylCoA dehydrogenase (HOAD; 1.1.1.35), lactate dehydrogenase (LDH; EC 1.1.1.27) and citrate synthase (CS; EC 2.3.3.1 previously 4.1.3.7) were essentially as described by (Suarez et al., 1986). Substrate concentrations were varied to ensure that they were saturating (not inhibitory). Activities of purified coupling enzymes were varied to ensure that their activities were in excess, i.e., not limiting to reaction rates. When necessary, aliquots of supernatant fractions were diluted in cold homogenization buffer to ensure linearity of rates. HK and lactate LDH were assayed in 50 mM HEPES buffer (pH 7.15) using samples homogenized in HEPES. To avoid inhibition by H^+ and ATP, PFK was assayed using samples homogenized in Tris and assayed in 50 mM Tris–Cl buffer at pH 8.35. β-Mercaptoethanol reacts with the DTNB used for CS and CPT assays and was not included in Tris buffers used for homogenization and assay (50 mM, pH 8.35). We found HOAD to be greatly inhibited by HEPES buffer, so this enzyme was assayed in 50 mM imidazole (pH 7.25) using samples homogenized in imidazole buffer. GP was assayed in 50 mM sodium phosphate (pH 7.4) using samples homogenized in imidazole buffer. Biochemical reagents and coupling enzymes were purchased from Sigma-Aldrich (USA); all other chemicals were from various sources and of the highest purity available commercially.

3. Results and discussion

3.1. $\dot{V}_{O_2}$ values during hovering

Whole body and mass-specific $\dot{V}_{O_2}$ values during hover-feeding at 'steady-state' (i.e., repeated feeding bouts have been occurring >30 min after fasting) are shown for each individual in Table 1. Most bats fed while hovering for less than 10 s; the longest bout recorded was 25 s. Over this wide range, there was no statistically significant relationship between hovering duration and $\dot{V}_{O_2}$ (data not shown). The mean $\dot{V}_{O_2}$/Mb of 21.0 mL O_2 g^{-1} h^{-1} is close to values reported by Winter et al. (1998) for nectar bats engaged in brief hovering bouts. This is about half the $\dot{V}_{O_2}$/Mb values displayed by small, hover-feeding hummingbirds (e.g., Lasiewski, 1963; Bartholomew and Lighton, 1986; Suarez et al., 1990) and similar to the maximal rates displayed by shrews exposed to low ambient temperature (Fons and Sicart, 1976).

3.2. Enzyme V_{max} values

The theory underlying the use of V_{max} values in metabolic studies such as this one has been outlined by Newsholme and Crabtree (1986). In the present work, we made use of an improved extraction technique that resulted in maximum solubilization of metabolic enzymes, as in Suarez et al. (1990) and Parra and Pette (1995). This change in tissue extraction protocol resulted in significantly higher activities of these enzymes (Suarez et al., 1990) compared with previously published values (Suarez et al., 1986).

Table 1
Average whole body ($\dot{V}_{O_2}$) and mass-specific ($\dot{V}_{O_2}/M_b$) oxygen consumption values for individual nectar bats (*Glossophaga soricina*) examined in this study.

Individual	Average $\dot{V}_{O_2}$ (mL O_2 h^{-1})	Average $\dot{V}_{O_2}/M_b$ (mL O_2 g^{-1} h^{-1})	*N*
1	257.22 ± 4.28	23.38 ± 0.39	4
2	236.27 ± 16.32	21.42 ± 1.54	3
3	200.73 ± 4.24	18.72 ± 0.58	4
4	219.44 ± 17.04	21.51 ± 1.67	3
5	215.26 ± 6.41	20.93 ± 0.72	6
6	219.16 ± 8.62	20.45 ± 0.67	6
7	232.76 ± 4.76	22.60 ± 0.46	6

Data are presented as mean ± SE. *N* = number of measurements for each individual.

Table 2
Maximum enzyme activities in nectar bat (*G. soricina*), hummingbird (*Selasphorus rufus*), rat (*Rattus norvegicus*), and shrew (*Blarina brevicauda*) skeletal muscles.

Enzyme	Nectar bat pectoralis	Hummingbird pectoralis	Shrew quadriceps[a]	Rat soleus
Glycogen phosphorylase	45.96 ± 0.72 (5)	59.0 ± 4.4 (5)[b]		10.08[c]
Hexokinase	15.94 ± 0.83 (5)	18.4[d]	1.1	2.20[e]
Phosphofructokinase	123 ± 16.29 (4)	109.8[f]		20.00[e]
Lactate dehydrogenase	345.52 ± 27.2 (5)	440.1 ± 49.1 (5)[b]	138.0	90.72[c]
Citrate synthase	204.66 ± 6.63 (5)	448.4[d]	37.0	45.08[c]
Carnitine palmitoyltransferase	6.00 ± 0.58 (5)	7.2[d]	2.7	0.28[e]
Hydroxyacyl-CoA dehydrogenase	134.1 ± 16.33 (4)	227.3 ± 18.5 (5)[b]	19.0	21.60[c]

Values are expressed in μmol min^{-1} g wet wt^{-1} and presented as means ± SE; number of animals in parentheses.
[a] From Stewart et al. (2005).
[b] Based on new measurements as described in Materials and methods.
[c] From Ardawi et al. (1989) obtained at 25 °C and expressed in units/g dry wt. Values are multiplied by 2 to correct for temperature and multiplied by 0.24 to express activity/g wet wt to allow estimation of v/V_{max} and to facilitate interspecific comparison (Scrutton and Utter, 1968).
[d] From Suarez et al. (1990). Enyzme activity originally reported for CPT was erroneously divided by 2, as in Crabtree et al. (1972a). Correct (non-divided) value representing CPT2 is reported here.
[e] From Blomstrand et al. (1983) obtained at 25 °C. Values are mutliplied by 2 to allow estimation of v/V_{max} and to facilitate interspecific comparison (Scrutton and Utter, 1968).
[f] From Suarez et al. (1986). PFK was found to be unstable in the frozen hummingbird muscles used in the present study.

Table 2 shows the V_{max} values measured in nectar bat pectoralis muscles alongside new as well as previously-published data from rufous hummingbirds. Freezing had no effect on hummingbird and nectar bat muscle enzyme activities, except in the case of PFK in hummingbird muscles, which lost most of its activity. Activities of all other enzymes measured were stable for at least a year in muscles frozen at −70 °C.

Data from the literature obtained from shrews (*Blarina brevicauda*), representing a small species of non-flying mammal, as well as from laboratory rats are included for comparison. Rat soleus contains abundant slow twitch, oxidative (type I) fibers, while shrew quadriceps consists of a mixture of fast-twitch, oxidative, (type IIa) and fast-twitch, glycolytic (type IIb) fibers (Suzuki, 1990). The much higher V_{max} values in nectar bat and hummingbird pectoralis muscles compared with shrew and rat muscles is immediately apparent. CS, a Krebs cycle enzyme often used as an indicator of relative mitochondrial content (Moyes, 2003), occurs in nectar bat pectoralis at activities about 46% of those in rufous hummingbird muscles. This reflects nectar bat mass-specific $\dot{V}_{O_2}$ values during hovering that are 40–50% of those reported in rufous hummingbirds. HK activities in nectar bats and rufous hummingbirds are similar and extremely high by avian and mammalian standards (e.g., Blomstrand et al., 1983). These high HK activities indicate increased capacities for flux at this step, given the regulatory role played by this enzyme in glucose metabolism in skeletal muscles (Fueger et al., 2004). Both hummingbirds and nectar bats possess pectoralis muscles consisting of fast-twitch, oxidative fibers (Grinyer and George, 1969; Hermanson et al., 1998) that are recruited for high-intensity, aerobic exercise. Accordingly, GP and LDH V_{max} values in both these species are much lower than those found in the locomotory muscles of many other vertebrate species capable of high-intensity, burst exercise, using musculature consisting exclusively (or mainly) of fast-twitch, glycolytic fibers (e.g., Guppy and Hochachka, 1978; Davis and Guderley, 1990).

The nectar bat and hummingbird CPT V_{max} values are 21- and 26-fold higher, while HOAD values are 6- and 10.5-fold higher, respectively, than those in rat soleus. The CS activities in nectar bat and hummingbird flight muscles exceed those in rat soleus by 4.5- and 10-fold, respectively, indicating higher mitochondrial content in flight muscles. This suggests that the high HOAD activities in flight muscles may simply be a consequence of their higher mitochondrial content. However, CPT activities in the flight muscles are much higher than would be expected on the basis of higher mitochondrial content alone, suggesting that these result from higher levels of enzyme gene expression. Given the evidence that CPT exerts control over fatty acid oxidation (Spurway et al., 1997; Eaton et al., 2001), the high activities of this enzyme in flight muscles indicate highly elevated capacities for flux through this pathway. Despite leading to more complete solubilization of mitochondrial enzymes, Triton X-100 has been found to inactivate CPT1 (Woeltje et al., 1987), the malonylCoA-regulated form of this enzyme (McGarry, 1995). Therefore, the nectar bat CPT activities reported here, as well as those we reported previously in rufous hummingbird flight muscle (Suarez et al., 1990), likely represent CPT2. This was validated by preliminary experiments (Suarez, unpublished) that showed the absence of malonylCoA inhibition of CPT activity in hummingbird pectoralis extracts prepared using Triton X-100. As a consequence, dividing by 2 to estimate the maximal capacity for flux, as in Crabtree et al. (1972a), based on the assumption that total activity represents the sum of activities of CPT1 and CPT2, is unnecessary and leads to the underestimation of flux capacities when dealing with samples homogenized with Triton X-100. Therefore, our CPT activities (Table 2) can be directly compared with estimated *in vivo* flux rates through fatty acid oxidation.

3.3. Substrate oxidation rates in vivo

Because during high-intensity, aerobic exercise, 90% or more of whole body $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ values are due to locomotory muscles (Taylor, 1987), these data combined with respiratory quotients ($RQ = \dot{V}_{CO_2}/\dot{V}_{O_2}$) estimated from them allow determination of the nature of the metabolic fuel(s) oxidized by muscles during exercise and the estimation of metabolic flux rates through the relevant pathways (Newsholme and Crabtree, 1986). Assuming that 26% of nectar bat body mass consists of flight muscle directly involved in power generation (Dudley and Winter, 2002) and that these account for 90% of whole-body $\dot{V}_{O_2}$, it can be calculated from Table 1 that, under steady-state conditions and when RQ = 1.0, the average O_2 consumption rate of per gram of flight muscle is 904.6 ± .1 μl min^{-1} or 40.39 ± 3.4 μmol O_2 min^{-1}. Given 2.41 ATP molecules synthesized/O atom consumed when glucose is oxidized (Brand, 2005), this corresponds to a rate of ATP turnover (i.e., synthesis = hydrolysis) during hovering of 194.66 ± 16.4 μmol g^{-1} min^{-1}. To sustain this rate of ATP turnover would require the oxidation of glucose at a rate of 6.73 ± .57 μmol g^{-1} min^{-1}. At the same rate of ATP turnover required to sustain hovering flight, the oxidation of fatty acid instead of glucose would require a rate of O_2 consumption 15% higher (Brand, 2005; Welch and Suarez, 2007). Assuming that fat stores fuel hovering, e.g., under fasting conditions, the rate of fatty acid oxidation, assuming palmitate as the sole substrate and a P/O ratio of 2.097 (Brand, 2005), would be 2.02 ± .17 μmol g^{-1} min^{-1}.

3.4. Enzymatic flux capacities and fractional velocities

In steady-state, the flux rates, *v*, through the individual enzyme-catalyzed reactions of glucose and fatty acid oxidation equal the overall pathway flux rates, J_{carb} and J_{fat}, respectively. Flux rates can be calculated from $\dot{V}_{O_2}$ values, based on the consumption of 12 O atoms in the oxidation of 1 molecule of glucose, and the consumption of 46 O

Table 3
Glucose and palmitate oxidation rates (expressed/g flight muscle) required to support hovering compared with maximum possible rates based on V_{max} values of hexokinase and CPT2, respectively, in the same individuals ($N=5$).

	Oxidation rate (μmol g^{-1} min^{-1})		
	Required	Maximum possible	v/Vmax
Glucose oxidation			Hexokinase
Rufous hummingbird	13.7	18.4	0.74
Nectar bat	6.21 ± 0.2	15.93 ± 0.84	0.39 ± .02
Palmitate oxidation			CPT2
Rufous hummingbird	3.8	7.2	0.53
Nectar bat	1.86 ± .06	6.00 ± 0.58	0.32 ± .04

Values are expressed in μmol min^{-1} g wet wt^{-1} and presented as means ± SE. Hummingbird glucose flux and hexokinase data are based on Suarez et al. (1990); CPT data are from Tables 1 and 2.

atoms in the oxidation of 1 palmitate molecule (Crabtree and Newsholme, 1972a,b; Brand, 2005). J_{carb}/V_{max} and J_{fat}/V_{max} represent the fractional velocities, i.e., v/V_{max} values, at which enzymes catalyzing nonequilibrium reactions operate (Suarez et al., 1997). In the case of HK, the J_{carb} values divided by the V_{max} values for HK measured in the same bats yield an average fractional velocity of 0.39 ± .02 ($n=5$); i.e., HK operates at 39% of its maximal capacity in hovering nectar bats if glucose serves as the sole substrate (Table 3). Assuming that palmitate is oxidized exclusively, J_{fat} divided by the V_{max} for CPT2 yields an average fractional velocity of 0.32 ± .04 ($n=5$); i.e., CPT2 operates at 32% of its V_{max} (Table 3). In comparison, HK and CPT2 in rufous hummingbird pectoralis operate at about 74% and 53% of their maximal capacities, respectively (Suarez et al., 1990).

The above calculations are possible because both hummingbirds and nectar bats can hover while displaying RQ values very close to the theoretical values corresponding to the oxidation of carbohydrate or fat (Suarez et al., 1990; Welch et al., 2008), therefore satisfying the required assumption of steady-state oxidation rates of either fuel. The results obtained further support the hypothesis that in both nectar bats and hummingbirds, high flux rates through these enzyme-catalyzed steps are achieved through high levels of enzyme expression, as well as regulatory mechanisms that lead to the operation of these enzymes at high fractional velocities. A limitation of our work is that we are unable to quantify the contribution of glycogen to flight metabolism. We expect that this is likely to be time-dependent and important in non-steady-state situations (e.g., transitions between rest and flight). In future research, we hope to address this issue using ^{13}C-nuclear magnetic resonance spectroscopy.

3.5. Comparison with other bat species

Yacoe et al. (1982) showed that, although the flight muscles of bats share a common metabolic design with other vertebrate fast and slow oxidative muscles, some of the interspecific variation observed in enzyme V_{max} values may be related to differences in diet. In particular, frugivorous bats possess significantly higher activities of HK than insectivorous bats, suggesting higher capacities for glucose utilization during flight among frugivores. In contrast, there seems to be no discernible pattern in the interspecific variation of GP activities among the bat species studied by Yacoe et al. (1982): *Artibeus lituratus* possesses higher GP activities than *Sturnira lilium*, although both are frugivores; GP activities in the latter species are about the same as in several species of insectivores. Relative to insectivorous bats, *Glossophaga soricina* pectoral muscles possess higher activities of both HK and GP, indicating higher capacities for utilization of both glucose and glycogen. The high capacity for glucose phosphorylation provides biochemical support for our previous finding that the oxidation of recently-ingested sucrose can provide most of the energy required for hover-feeding flight in these nectar bats (Welch et al., 2008). The high HK activity is not a unique feature associated with nectarivory but, instead, is a trait shared with frugivorous bats (Yacoe et al., 1982). Clearly, the evolution of physiological and biochemical traits associated with nectarivory in Phyllostomid bats is worthy of further study.

3.6. Convergence with hummingbirds

Hummingbirds are capable of prolonged hovering; Lasiewski (1962), for example, published $\dot{V}_{O_2}$ data obtained from individuals that hovered 35 and 50 min in a metabolic chamber. Although nectar bats can be induced to hover-feed for several seconds in the laboratory, Voigt and Winter (1999) observe that they tend to do so for 1 s or less in the wild. Their maximum record of 17 s and our report here of a bout lasting 25 s supports their conclusion that the basic structure of glossophagine bats supports hovering flight. Nectar bat pectoralis muscles lack fast-twitch, glycolytic fibers and consist of two types of fast-twitch, oxidative fibers (Hermanson et al., 1998). That hovering flight involves aerobic exercise is supported by the finding that, in fasted bats, it is fueled by fatty acid oxidation (Welch et al., 2008), an aerobic pathway that, at a given power output, requires an even higher rate of O_2 consumption than carbohydrate oxidation (Welch and Suarez, 2007). In addition, after hovering, nectar bats immediately resume forward flight and do not need to rest to recover from fatigue.

When the control of flux is shared by multiple reactions in linear pathways, metabolic control analysis reveals that the design of pathways for high flux rates requires the concerted up-regulation of capacities at multiple steps (Fell and Thomas, 1995; Fell, 1997). Control analysis, applied to whole body O_2 (Jones, 1998) and metabolite (Brown, 1994) fluxes, reveals that control is shared by multiple steps even at higher levels of organization. The picture that emerges in the case of hovering, nectarivorous vertebrates is consistent with the idea that sugar fluxes from intestines to catabolic pathways in muscles are subject to regulation at multiple steps, and that the up-regulation of flux capacities at various steps has evolved to support their high energetic requirements. High intestinal sucrase activities (Hernández and Martínez del Rio, 1992; McWhorter and Martinez del Rio, 2000), plus the presence of both active transport and passive mechanisms for hexose sugar movement across the intestinal epithelium (McWhorter et al., 2006; Caviedes-Vidal et al., 2008) are found in both hummingbirds and frugivorous bats, and it seems likely that nectar bat intestines would possess active and passive transport mechanisms, as well. In the flight muscles, we find high enzymatic capacities for flux in pathways of glucose and long-chain fatty acid catabolism, as well as high capacities for mitochondrial oxidative metabolism (Suarez et al., 1990; Suarez et al., 1991; Table 2). The entire cardiorespiratory system is geared to supply O_2 at phenomenally high rates to the abundant muscle mitochondria in small bats and hummingbirds (see reviews by Suarez, 1992; Maina, 2000), enhancing capacities for convective and diffusive fluxes of both O_2 and metabolic fuels. However, hummingbirds possess lungs with higher O_2-diffusing capacities than mammalian lungs (Dubach, 1981). Nectar bats possess longer wings than hummingbirds of similar mass, reducing the amount of induced power required to support hovering flight (Voigt and Winter, 1999). The resulting lower mass-specific $\dot{V}_{O_2}$ values (and, therefore, lower carbon flux rates) during hovering (Table 1; Winter et al., 1998; Winter and von Helversen, 1998) leads to lower fractional velocities at the HK and CPT2 reactions in nectar bat compared with hummingbird flight muscles. Despite these differences, data from both hummingbirds and nectar bats support the hypothesis that, at the biochemical level, high metabolic flux rates are made possible by high concentrations of enzymes that catalyze nonequilibrium reactions and the operation of these enzymes at higher fractional velocities than in other species (Suarez et al., 1990; Suarez et al., 1997).

The fraction of energy requirements that can be fueled by recently-ingested dietary sucrose during hovering (Welch et al., 2008) is also lower in nectar bats than in hummingbirds. In nature, the nectars provided by bat-visited flowers are richer in monosaccharides than the nectars of hummingbird-visited flowers (Baker et al., 1998). It is

possible that nectar bats are capable of fueling close to 100% of their flight metabolism when provided mixes of simple sugars that mimic those found in their natural diets.

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