REVIEW
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The Metabolic Flexibility of Hovering Vertebrate Nectarivores

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Foraging hummingbirds and nectar bats oxidize both glucose and fructose from nectar at exceptionally high rates. Rapid sugar flux is made possible by adaptations to digestive, cardiovascular, and metabolic physiology affecting shared and distinct pathways for the processing of each sugar. Still, how these animals partition and regulate the metabolism of each sugar and whether this occurs differently between hummingbirds and bats remain unclear.

Introduction

The task of achieving energy homeostasis is an especially challenging one for vertebrate pollinators like hummingbirds and small bats. Flight requires the highest rates of metabolic power input of any form of locomotion, and these smallest fliers employ the most energetically intensive form of flight: hovering (78, 97, 102). Small body size (≈2–20 g for hummingbirds; ≈10–30 g for nectar bats) and endothermy mean that these animals must often sustain high rates of metabolism even during inactive periods, especially when ambient temperatures fall below an animal's thermoneutral zone. Even more impressive, several hummingbird and bat species are long-distance migrators, capable of sustaining energetically expensive migratory flight for extended periods, exclusively fueled by onboard fat stores (22, 59).

The nectars (and, for bats, fruits) that hummingbirds and nectar bats rely on for most of their caloric intake present a readily digestible, energy-rich resource (58). During foraging periods, these animals visit flowers (or consume fruits) at regular intervals, ensuring continuous ingestion of sugars. It seems obvious, even to the casual observer of a backyard hummingbird feeder, that these animals "run on nectar sugar." However, among well-studied mammalian species, constraints to dietary sugar ingestion, absorption, and oxidation limit extensive reliance on ingested sugar as a fuel for ongoing exercise (30, 31). Thus, even if the ability of hummingbirds and bats to rely extensively on ingested sugar as a fuel is intuitively satisfying, it is a remarkable metabolic feat indicative of a remarkable underlying physiology. Still, given that carbohydrate energy stores (e.g., circulating blood sugars, hepatic or intramuscular glycogen) are energy sparse, they are not an ideal fuel store for fasting fliers. Hence, these animals must possess the ability to convert ingested sugars to a more energy-dense storage form (fat) and amass these energy stores at rates sufficient to build large reserves capable of seeing them through fasting periods, even when energy turnover might remain relatively high.

In the following review, we examine the challenges hummingbirds and nectar bats face in using nectar sugars to both fuel immediate energy demands as well as amass energy reserves for use during non-foraging periods. We characterize physiological strategies that these animals rely on to ensure rapid uptake and oxidation, or storage of dietary carbon, with an emphasis on the possible distinct handling of each principal nectar sugar: glucose and fructose. Last, we identify important gaps in our understanding of the physiological mechanisms that regulate sugar use as an oxidative or lipogenic fuel and highlight differences in avian and chiropteran physiology that imply distinct strategies used by each group to achieve the same fuel use phenotype.

Aerial Refueling: Nectar Sugar Fuels Foraging Activity

Patterns of fuel use during exercise are highly conserved among non-flying mammals (52), with low-intensity exercise supported primarily by lipid oxidation with a shift toward primary reliance on oxidation of intramuscular glycogen at high intensities (10, 52, 92). Proportionate reliance on recently ingested or circulating blood sugar peaks at relatively low exercise intensities (27, 32, 70, 90) and accounts for, at most, 35% of overall metabolic fuel use (29, 92). Although birds do rely to a variable extent on endogenous carbohydrates as a fuel for flight when not fasted (23), long flights, or flights by fasted birds, are fueled by oxidation of onboard lipid stores (11, 22, 91). Thus, although hummingbirds and nectar bats have a sugar-rich diet, a priori expectations of high rates of dietary sugar oxidation during foraging are without precedence among other vertebrates studied.

To investigate fuel use in hummingbirds, Suarez and colleagues (82) monitored respiratory exchange ratios (RER = $\dot{V}CO_2/\dot{V}O_2$; rates of CO_2 consumption/

O_2 consumption) and deduced that hummingbirds initially oxidized lipids during the first hover-feeding following a fast (i.e., that the RER ≈0.7). The team then observed a rapid increase in RER values with each subsequent feeding event, with RER ≈1.0 after only several minutes, indicative of a switch to carbohydrate oxidation (82). Following from this work, Welch, Suarez, and collaborators, published a series of papers combining feeder mask respirometry with a diet-switching carbon stable isotopic tracer approach (technique reviewed in Refs. 53, 100) to show that this change in RER was commensurate with a switch from oxidation of endogenous (lipid) carbon stores to newly ingested nectar sugar (80, 95, 98). Not only was this switch in fuel use comparatively rapid, but hummingbirds appeared able to fuel up to 100% of energetically expensive hovering flight with either glucose or fructose ingested only minutes prior, achieving much greater proportionate (FIGURE 1A) and absolute (FIGURE 1B; Table 1) rates of dietary or circulating sugar flux during exercise than that seen in humans (32), rodents (20, 63), or other cursorial mammals (92). Subsequently, approximately simultaneous work utilizing similar approaches two teams showed that nectar bats, existing on a similarly specialized sugar-rich diet, exhibited qualitatively identical patterns of fuel use during foraging (Refs. 89, 97; FIGURE 1A).

This work revealed two important facts: unlike in most mammals, fuel use in vertebrate nectarivores is determined by dietary status and not by exercise intensity; unlike in any other vertebrate group examined, apparent rates of uptake and oxidation of fructose were equal to that for glucose (FIGURE 1). This second finding is especially intriguing, since it both implies a metabolic flexibility that other animals do not possess and raises interesting questions regarding whether and how nectarivores might regulate and partition the metabolism of each sugar species.

Bottlenecks to the flux of glucose from diet to exercising muscles exist at multiple steps, including the hydrolysis of sugar polymers in the intestine, hexose absorption across the intestinal brush border, and uptake and phosphorylation by end-use tissues (reviewed in Refs. 32, 70, 90). Previous studies identified multiple adaptations, common to most flying vertebrates, that permit exceptionally high rates of oxygen flux from the environment to their muscle mitochondria (27, 48, 78). Work on hummingbirds and nectar bats now suggested there were homologous adaptations to digestive, cardiovascular, and metabolic physiology that enable the highest rates of carbon (sugar) flux from the environment (nectar or fruit pulp) to the same muscle mitochondria (80, 84). Although it is a more nascent field of research than that seeking to understand variation in the "oxygen transport cascade" (93) underlying variation in aerobic exercise capacity, recent progress in our understanding of variation in the analogous "sugar oxidation cascade" (80) has been made. This is summarized below.

Rapid Sugar Digestion and Absorption

Once ingested and passed to the intestine, the initial key regulatory step in the "sugar oxidation cascade" involves hydrolysis of complex carbohydrates and disaccharides to their component monosaccharides, followed by their uptake across the intestinal brush border. Sucrase hydrolyzes sucrose yielding fructose and glucose, and its expression correlates with each group's typical diet. Proportions of sucrose, glucose, and fructose in nectars vary interspecifically (3, 69), with hummingbirds tending to consume nectar high in sucrose (>50%; Ref. 5), whereas nectar bats typically consume nectars and fruits lower in sucrose (<50%) and higher in glucose and fructose (4). Hummingbirds have one of the highest sucrase activities measured in any vertebrate, in contrast with lower activities seen in passerines (49, 71). Nectar bats have comparatively lower sucrase activity than hummingbirds but similar activity to levels in fruit bats, likely reflecting the lower proportion of sucrose in the bat diet (25, 26). Despite having lower sucrase activity than hummingbirds, sucrase does not appear to limit the digestive efficiency of nectar bats consuming 1 M sucrose diets (2).

Similar to other aerial vertebrates, hummingbirds and nectar bats have shorter guts, with reduced surface area compared with similar-sized terrestrial mammals (15). Thus flying nectarivores must paradoxically meet comparatively higher energy demands despite more rapid gut transit times, less absorptive area, and higher dietary intake (65). Cellular-mediated absorption of glucose and fructose across the intestinal brush border in hummingbirds and bats occurs via sodium-glucose cotransporter 1 (SGLT1) and glucose transporter 5 (GLUT5), respectively (87, 104). The intestinal surface area-specific rates of uptake in hummingbirds are among the highest known (37), whereas the rates in bats are unremarkable compared with similarly sized terrestrial mammals (36).

Nevertheless, capacities for cellular-mediated sugar uptake in hummingbirds and nectar bats may be insufficient to account for the observed sugar assimilation efficiencies of >95% (37, 38), particularly when ingestion rates are high. Like other flying vertebrates, hummingbirds and nectar bats also employ paracellular absorption of nutrients: the passive absorption of small nutrients

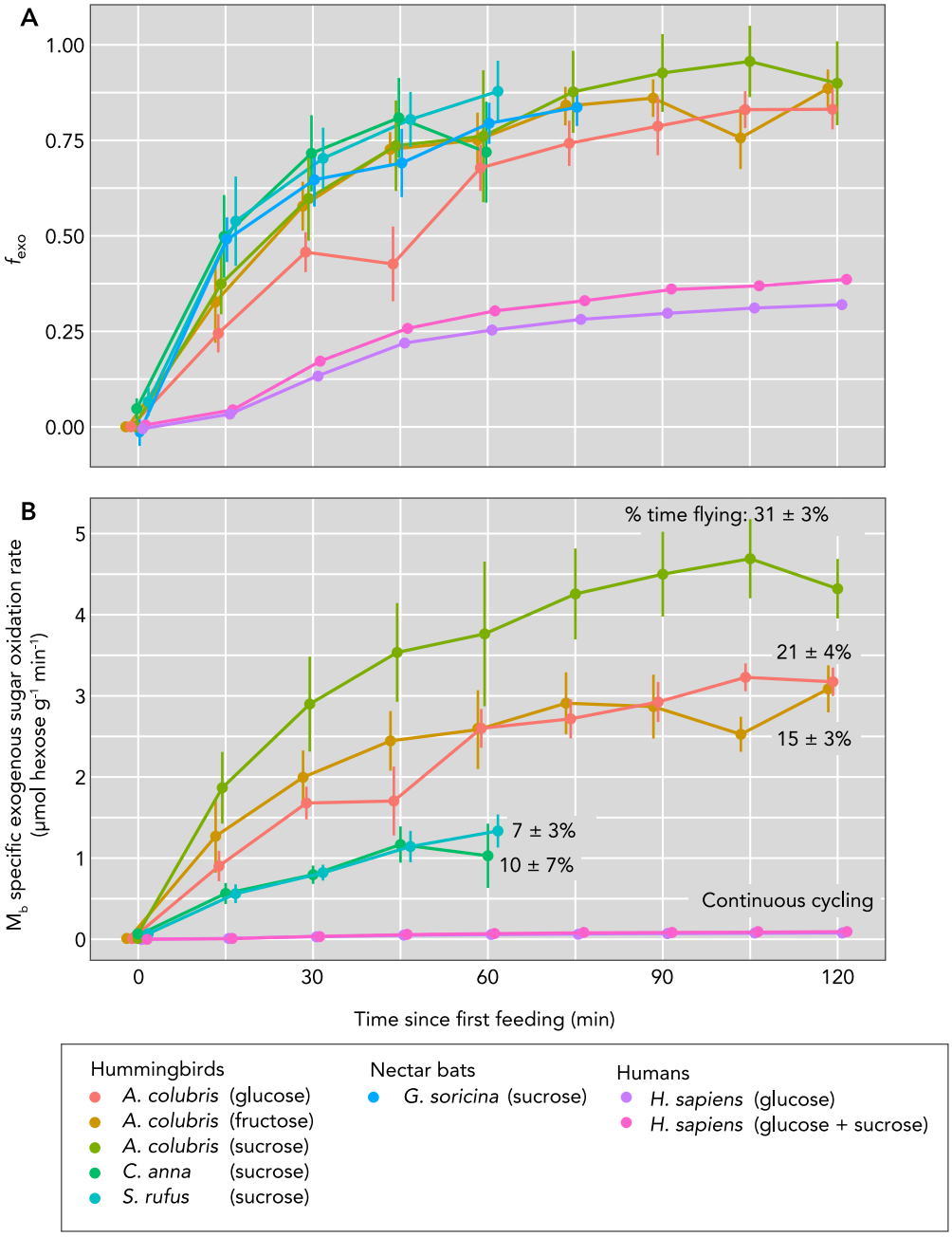

FIGURE 1. **Vertebrate nectarivores oxidize newly ingested sugars at comparatively high rates**

Specifically, hummingbirds and nectar bats oxidize ingested nectar sugars to support a higher proportion of exercise metabolism (*A*), and at greater mass-specific rates than in humans and other vertebrates (*B*). *A*: f_{exo}, the proportion of exhaled CO_2 resulting from oxidation of isotopically labeled, newly ingested (exogenous) sugars during foraging or exercise. *B*: calculated body mass (M_b)-specific rates of newly ingested (exogenous) sugar oxidation (μmol·g^{-1}·min^{-1}), during foraging or exercise. Data are shown for three species of hummingbird (ruby-throated, *Archilochus colubris*; Anna's, *Calypte anna*; and rufous hummingbird, *Selasphorus rufus*), one species of nectar bat (Pallas' long-tongued nectar bat, *Glossophaga soricina*) and humans (*Homo sapiens*). Hummingbirds and bats performed hover-feeding bouts at will, interspersed by periods of perching, and humans exercised on a cycle ergometer at 50% of maximal aerobic rate. Rates of exogenous sugar oxidation in hummingbirds were calculated based on time/energy budgets, and rates of oxidation were generally positively related with foraging effort (% of time spent flying/hover-feeding, as indicated). Because time budgets were not recorded in bats, these data are not available. Data are plotted in relation to time since the start of the foraging or exercise period. Hummingbirds, bats, and humans were fasted before the start of the experiment. Data redrawn from Refs. 16 (*A. colubris*), 98 (*C. anna* and *S. rufus*), and 29 (*H. sapiens*). For additional methodological details, please consult the cited work.

Table 1. *Trial hexose oxidation rates*

	S. rufus	*C. anna*	*A. colubris*			*G. sorcina*	*H. sapiens*	
	Sucrose (n = 4)	Sucrose (n = 3)	Sucrose (n = 5)	Glucose (n = 6)	Fructose (n = 6)	Sucrose (n = 7)	Glucose (n = 9)	Glucose + sucrose (n = 9)
Body mass, g	3.71 ± 0.12	4.98 ± 0.48	2.90 ± 0.13	2.99 ± 0.15	2.93 ± 0.11	10.2 ± 0.1	74,100 ± 1,900	74,100 ± 1,900
Trial hexose oxidation rate, μmol hexose g^{-1} min^{-1}	0.50 ± 0.05	0.52 ± 0.15	2.84 ± 0.17	1.49 ± 0.15	1.66 ± 0.16		0.078 ± 0.004	0.089 ± 0.004
Time spent hovering, %	6.6 ± 3.4	9.6 ± 7.4	31 ± 3	21 ± 4	15 ± 3			
Hovering hexose oxidation rate, μmol hexose g^{-1} min^{-1}	3.29 ± 0.26	3.05 ± 0.46	6.40 ± 0.52	4.63 ± 0.40	4.46 ± 0.24	2.04 ± 0.11		
t_{50}, min	6.7 ± 0.9	12.2 ± 0.4	16.9 ± 1.4	13.3 ± 2.2	12.4 ± 1.7	9.9 ± 1.9	29	27
Refs. data is based on	98	98	16	16	16	97	29	29

Values are means±SE. Hovering nectarivores have higher mass-specific rates of exogenous hexose oxidation compared with humans. Trial hexose oxidation rates for rufous (*S. rufus*), Anna's (*C. anna*), and ruby-throated (*A. colubris*) hummingbirds incorporate time-energy budgets (% hover feeding vs. perching), and continuous cycling at 50% maximal aerobic rate in humans (*H. sapiens*). Exogenous hexose oxidation rates increase to meet energy demands during a hovering bout. Time-energy budgets are not available for Pallas' long-tongued nectar bat (*G. soricina*), and only hovering rates are reported. t_{50}, time at which 50% of carbon isotopes are exchanged in animals breath, and is calculated from kinetics of "disappearance" of labeled carbon (k_d, sensu 99) in hummingbirds, and "appearance" (k_i values) in humans.

across comparatively leaky tight junctions binding adjacent enterocytes (reviewed in Ref. 66). This provides a rapid and low-cost means of absorbing molecules and accounts for the majority of hexose absorption in small aerial vertebrates (42), including hummingbirds (55) and nectar bats (68). The proportion of active and passive absorption in vivo is difficult to determine in hummingbirds due to their small size. However, paracellular absorption provides flexibility, and the proportion of paracellular absorption increases with nectar concentration (55). In the case of nectar bats, paracellular sugar absorption is not only sufficient but required to fuel hovering flight with recently ingested sugars (68).

Circulatory Delivery of Sugars

Because they are both transported via the circulatory system, many of the adaptations in the "oxygen transport cascade" that enhance oxygen delivery simultaneously enhance the delivery of glucose, and potentially fructose (80). Rates of oxygen and sugar delivery to tissues are a function of cardiac output and blood oxygen or sugar levels, and are enhanced by higher capillary volume densities, which reduce diffusion or transport distances. Hummingbird heart rates during flight range between 480 and 1,200 beats/min (BPM) (18, 41), and their cardiac output is approximately five times their body weight per minute (33). Hematocrit, an indirect measure of oxygen-carrying capacity, is also high, at 56.3% (34). Bats generally also exhibit enhanced cardiac output and oxygen-carrying capacities. Frugivorous tent-making bat (*Uroderma bibobatum*) heart rates have been recorded reaching upward 900 BPM during flight (60). Egyptian fruit bats (*Rousettus aegypticus*) exhibit hematocrit values as high as 55%, greater than in similarly sized non-flying mammals such as shrews (39–50%; Refs. 35, 73). Both hummingbirds (6) and nectar bats (25 mM; Ref. 39) exhibit exceptionally high postprandial blood glucose levels compared with similarly sized terrestrial mammals. Electron micrograph analysis of hummingbird flight muscle reveals a two to six times higher capillary volume density compared with mammals (50), and although unreported in nectar bats, capillary volume density is high in insectivorous bats (51). Collectively, it is clear that glucose delivery to tissues is highly enhanced in these aerial nectarivores. Frustratingly, blood fructose levels are unreported in any of these groups. Thus similar conclusions about fructose delivery capacity remain elusive.

Oxygen and the carbon in dietary sugars converge in the mitochondria of aerobically active tissues. Thus the mitochondria, as end consumers of both oxygen and sugar carbon, play a key role in establishing the overall flux of each. Unsurprisingly, both nectar bats and hummingbirds exhibit exceptionally high activities of mitochondrial enzymes such as citrate synthase (83). Both structural and enzymatic properties of hummingbird mitochondria contribute to the increased rate of substrate utilization observed (50). Hummingbird mitochondria occur at densities near theoretical physiological maximums, comprising 35% of overall muscle fiber volume (81). Although not yet directly demonstrated in nectar bats, high mitochondrial abundance is unsurprisingly the case in bats in general (51), since they all employ energetically expensive flight to forage.

Rapid Sugar Transport Into Tissues

As in cellular-mediated sugar uptake in intestinal brush-border cells, sugar transport across other cell membranes requires facilitated transport through glucose transporters (GLUTs). In mammalian

muscle, several GLUT isoforms, including GLUT1 and GLUT3, are expressed at low levels, supporting low capacities for glucose uptake (21). GLUT4, expressed at relatively higher levels, is important to overall glucose uptake capacity in muscle and to overall blood glucose regulation. In response to elevated blood glucose, peripheral tissues, including the muscle of most vertebrates, translocate GLUT4 from intracellular vesicles to the sarcolemma, transiently increasing the uptake capacity for glucose (61, 75). Indeed, this response is a highly conserved feature of the insulin-mediated blood glucose regulatory program. This mechanism is enhanced in nectar bat flight muscle by comparatively high densities of GLUT4 (FIGURE 2C; Ref. 84), suggesting a relatively high capacity for glucose uptake. Exercise, which independently stimulates GLUT4 translocation (61), is thought to be an important regulator of blood glucose in nectar bats (39). This conclusion was inferred by noting that the rapidity with which high (≤25 mM) postprandial blood glucose levels returned to prefeeding levels was positively correlated to the level of flight activity (39).

Unlike all other vertebrate taxa, birds do not possess a GLUT4 gene (FIGURE 2C; Refs. 9, 14, 64, 74, 86, 103). Consequently, blood glucose concentrations of birds are unresponsive to physiologically relevant concentrations of insulin (9). Given GLUT4's importance in enabling glucose uptake capacity in the muscles of all other vertebrates, its absence in hummingbird flight muscle is striking. What transporter(s) imbue hummingbird flight muscle with high apparent capacities for glucose uptake? In contrast with most vertebrates, hummingbird flight muscle expresses relatively high abundance of GLUT1 transcript, suggesting substantial GLUT1-mediated glucose uptake capacity (FIGURE 2A; Ref. 57). GLUT3 transcript has been observed in hummingbird muscle, although its

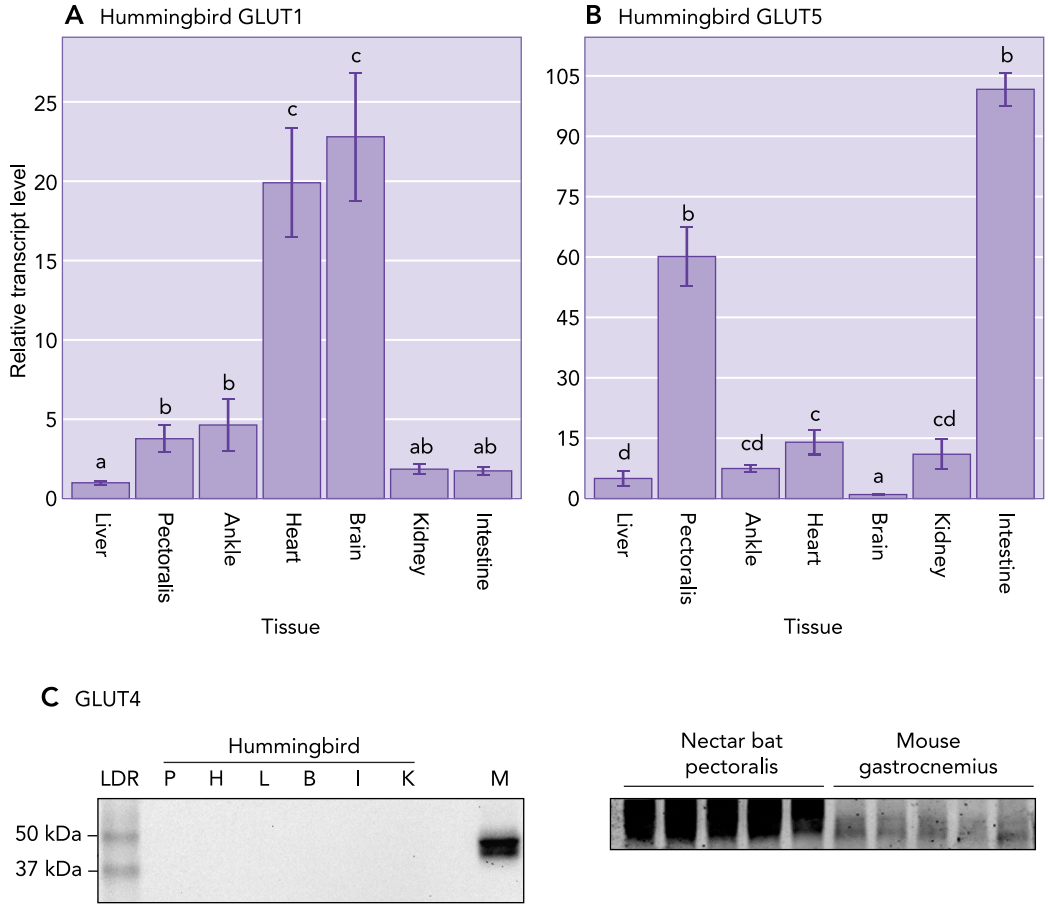

FIGURE 2. **Glucose transporter expression patterns in vertebrate nectarivores**
Elevated expression of glucose transporters may underlie exceptional rates of sugar flux into splanchnic tissue and flight muscle in hummingbirds, and implies differences between hummingbirds and nectar bats with respect to how sugar flux is maintained and regulated. Relative transcript abundance of glucose transporter GLUT1 (*A*) and fructose transporter GLUT5 (*B*) among tissues of the ruby-throated hummingbirds (*A. colubris*). Expression is based on qPCR data normalized to Elf1α1, and redrawn from Ref. 57 with permission. Different letters indicate tissues with significantly different levels of expression based on Tukey multiple comparisons. *C*: Western blots showing absence of GLUT4 expression in ruby-throated hummingbird flight muscle (pectoralis, P), heart (H), liver (L), brain (B), intestine (I), and kidney (K) tissue (LDR, ladder; M, mouse heart positive control; reprinted from Ref. 94 with permission), and relatively high expression in nectar bat (*G. soricina*; reprinted from 84 with permission) flight muscle.

contribution to glucose uptake capacity remains unknown because neither transcript nor protein abundance has been quantified (94). Similarly, GLUT1 and GLUT3 abundance has not been assessed in nectarivorous bats.

In most vertebrates, skeletal muscle has very little fructose uptake capacity because fructose-specific isoforms (e.g., GLUT5) are expressed at low levels (21, 28), and isoforms that transport both glucose and fructose (e.g., GLUT2) are absent (87). Hummingbird flight muscles have relatively high transcript abundance of GLUT5, exceeding that of kidney and comparable even to transcript abundance in intestine (FIGURE 2B), both tissues with much higher relative GLUT5 abundance than other tissues in all mammals examined (1, 5, 66, 105). If transcript abundance is indicative of protein abundance for this gene, then capacity for uptake of fructose into flight muscle may be very rapid compared with other vertebrate muscles. Whether an analogous adaptation exists in nectar bats remains to be elucidated. Thus, although new evidence provides tantalizing clues, much work needs to be done to understand the molecular basis of apparent high-glucose and fructose-uptake capacities in vertebrate nectarivore flight muscle.

Rapid Sugar Oxidation in Tissues

Following uptake into muscle or other tissues, both glucose and fructose must be phosphorylated to direct them to a further catabolic or anabolic fate, to trap the sugar in the cell, and to maintain a concentration gradient for GLUT-mediated uptake (101). Capacities for rapid phosphorylation of glucose by hexokinase, as estimated by apparent V_{max}, are four to eight times as rapid as that observed in mouse soleus muscle (8, 82, 83) and exceed calculated rates of glucose flux through glycolysis in vivo (83). This is a key difference compared with "aerobic" terrestrial vertebrates, who are unable to sustain high glycolytic flux using circulating glucose and are dependent on intramuscular glycogen (92).

The apparent ability of hummingbirds to sustain foraging when offered a fructose solution (16) implies that fructose must not only be taken up but must also be phosphorylated by flight muscle at high rates. Although glucose is readily phosphorylated by hexokinase in model mammalian species (101), known hexokinases have comparatively low affinity for fructose (12), and most fructose is taken up by the liver and kidneys, where it is phosphorylated by ketohexokinase, the first enzyme of the fructolysis pathway (85). As in other vertebrates (17), key regulatory fructolytic enzymes ketohexokinase and aldolase B are transcribed at only low levels in hummingbird muscle (57), paradoxically implying low fructolytic capacity through this pathway. However, although apparent capacities for phosphorylation of fructose by flight muscle hexokinase are not as rapid as they are for glucose, they are still over three times more rapid than rates observed with glucose in mouse soleus muscle (8, 57). Although V_{max} values for hexokinase-mediated phosphorylation of fructose are lower than calculated rates of apparent fructolysis and oxidation in hummingbird flight muscle during hovering (96), they do exceed rates of apparent fructolysis when these are averaged over the entire foraging period (bouts of foraging flight separated by periods of perching; Ref. 57). Thus it seems plausible that direct fructose phosphorylation in hummingbird flight muscle, temporally buffered, for example, by the oxidation of hepatically generated fructolytic metabolites (e.g., lactate, pyruvate, or glucose), could support ongoing foraging activity. Capacities for fructose phosphorylation (i.e., fructolytic enzyme activities or apparent hexokinase-mediated phosphorylation in muscle) in nectar bat flight muscle remain unknown. Available evidence suggests that nectar bats, like hummingbirds, can oxidize fructose at high rates to support foraging, although much work remains to be done to clarify the role of fructose as an oxidative fuel in this group.

Oxidation vs. Lipogenesis: Distinct Fates for Component Nectar Sugars?

Circulating sugars are, like the flowers from which these nectarivores obtain their food, ephemeral in nature. In both hummingbirds and bats, isotopic tracer studies indicate that the turnover of ingested sugar molecules in the pool of actively metabolizable substrates is rapid. The time necessary for a 50% turnover of ingested sugar molecules within the metabolizable pool (13) is $<$15 min in hummingbirds and nectar bats, whereas in humans it is roughly 30 min (88).

As noted above, fasted hummingbirds and nectar bats fuel energetically expensive flight by oxidizing onboard lipid stores (16, 97, 98). Since lipids do not generally comprise a substantial portion of ingested calories in the hummingbird diet, endogenous lipid reserves must be synthesized de novo from ingested sugars. Because energy demands during fasting periods may be quite high (such as during migratory flights or overnight periods at low ambient temperature), these animals must possess the ability to rapidly build expansive energy stores via de novo lipogenesis (DNL).

The importance of dietary sugars in promoting DNL, obesity, and metabolic disorders such as diabetes in humans is hotly debated (46, 47, 67, 76). Although evidence from studies in rodents

chronically fed a high-carbohydrate diet indicate that DNL from sugar precursors accounts for 60–70% of circulating fatty acids (56), evidence in human studies is more equivocal. Recent reports indicate DNL accounts for between <5% (62) to >12% (72) of circulating triglycerides in human subjects fed a high-carbohydrate, low-fat diet.

Due in part to its preferential uptake and metabolism by splanchnic tissues like the liver, fructose is hypothesized to stimulate hepatic DNL to a greater extent than glucose (43). Fructose has been shown to raise triglyceride levels in humans, but this effect is only consistently observed in healthy adults when fructose intake is exceptionally high (>95th percentile intake rates compared with average U.S. intake rates) and is coupled with elevated total caloric intake rates (45). Sievenpiper et al. (76) suggest that the inclusion of supraphysiological doses of fructose typically included in "high-carbohydrate" diets fed to rodents may partly explain the apparent greater effect of these diets on DNL in rodent systems. Unlike for most vertebrates, fructose is abundant in the nectarivore diet, raising interesting possibilities regarding the extent to which hummingbirds and nectar bats utilize glucose vs. fructose for DNL.

The liver is considered the primary lipogenic tissue in birds (7, 24), and hummingbird livers are hypothesized to possess the greatest biosynthetic capacity of any vertebrate hepatic tissue (79). This hypothesis derives from the exceptional activity of both enzymes crucial to gluconeogenesis (e.g., pyruvate carboxylase) and fatty-acid synthesis (e.g., acetyl-CoA carboxylase; Ref. 79). Given that the hummingbird liver abundantly expresses fructolytic enzymes (e.g., ketohexokinase and aldolase B), it is likely that this tissue is adept at metabolizing fructose (57). Following from the observation that the liver is a principal site of fructose metabolism in humans and rodents (85), it is possible that fructose is preferentially metabolized in the hummingbird liver, sparing glucose for direct oxidation by active tissues such as heart and flight muscle, as well as glucose-dependent tissues like brain. This hepatic metabolism of fructose in hummingbirds may convey substantial metabolic flexibility, allowing the rationalization of observed patterns of fuel use (16, 80, 98). A theoretical metabolic framework illustrating possible partitioning and highlighting pathways for rapid uptake and metabolism of both glucose and fructose are shown in FIGURE 3. For example, when fructose is ingested by itself (e.g., Refs. 16, 89), some circulating fructose may be directly oxidized in flight muscle and heart, whereas some may be used for hepatic DNL and gluconeogenesis, with the liver becoming a net glucose, lactate, and/or pyruvate exporting organ (16). In contrast, when glucose and fructose are ingested together (as sucrose or mixed monosaccharides), glucose may preferentially be directly oxidized in active tissues (muscle, heart, brain), with fructose preferentially directed toward DNL and, to a lesser extent, the production and export of glucose, lactate, and pyruvate.

Comparatively less is known about the extent to which nectar bats build fat stores via DNL or do so in the liver vs. adipose tissue (54). The biosynthetic capacity of nectar bat liver is yet to be characterized but likely exhibits similar enzymatic adaptations to those characterized in hummingbirds (79). Furthermore, nectar bats may dramatically increase insect intake during some seasons, obtaining substantial lipids from their diet. Thus much work remains to be done to understand differences in lipid storage and usage between avian and chiropteran lineages (54).

Still other metabolic fates are possible for each sugar. For example, the contribution of the pentose phosphate pathway (PPP) as a potential route for glucose or fructose catabolism is unclear. The first step of the PPP is glucose-6-phosphate dehydrogenase, cleaving the first carbon of the glucose molecule. This produces NADPH and CO_2 and a ribose sugar that can enter glycolysis or be used in nucleotide and amino acid synthesis (77). The CO_2 released can lead to a RER of >1 and could represent production of NADPH to support DNL (19). Recently, an alternative explanation for the increase in RER and use of NADPH has been proposed in nectarivores using a hawkmoth model (*Manduca sexta*; Ref. 44). Levin et al. (44) proposed that, between flight bouts, glucose (or possibly fructose) is shunted through the PPP and that the NADPH produced is used in the regeneration of the antioxidant glutathione. The remaining ribose sugar is then oxidized during flight. High antioxidant capacity may be critical for hovering animals because associated high metabolic rates may increase reactive oxygen species generation. Rapid diversion of hexose from glycolysis to the PPP can occur with acute oxidative stress and can be a key step in maintaining redox balance (40). Whether hummingbirds and other nectarivores partition either glucose or fructose through the PPP to maintain the glutathione pool and manage oxidative stress is unknown but interesting given the high metabolic rates and low dietary antioxidants increasing the demand on endogenous antioxidants.

Conclusions and Future Directions

Studies tracking fuel use in hovering hummingbirds and nectar bats unequivocally demonstrate an exceptional capacity for and flexibility in reliance on either endogenous lipid or on the glucose

or fructose components of their nectar diets (16, 80). Studies intended to understand the mechanistic basis of high aerobic capacity in these groups have revealed adaptations that simultaneously enhance both oxygen and sugar flux from the environment to active flight muscles (80, 84). Yet, although tantalizing clues now exist regarding how sugar uptake across tissue borders is enhanced in these groups (e.g., GLUT mRNA expression patterns), many questions remain. For example, although exceptional GLUT4 protein levels may be present in nectar bat flight muscle, potentially underlying high capacities for glucose uptake (84), the basis for high fructose uptake capacity in flight muscle is unclear in nectar bats and far from proven in hummingbirds.

In switching almost completely between lipid and sugar oxidation, hummingbirds and nectar bats must acutely regulate fuel use at a tissue and systemic level. The lack of GLUT4 (and an associated insulin-mediated response) in hummingbirds means that some aspects of glucose use must differ between each group. Indeed, although both nectar bats and hummingbirds experience relatively high postprandial peaks in blood glucose, fasting values differ significantly. Nectar bats regulate fasting blood glucose levels at ~5 mM, similar to terrestrial mammals, including humans (39). Hummingbirds, in contrast, exhibit much higher fasted blood glucose levels (~17 mM; Ref. 7), like other birds generally, although to an extreme (9). Just as important, almost nothing is known about how fructose metabolism, either directly in flight muscle or via splanchnic tissue, is controlled in either group. We call for work to be done to understand how metabolism of each sugar type is controlled

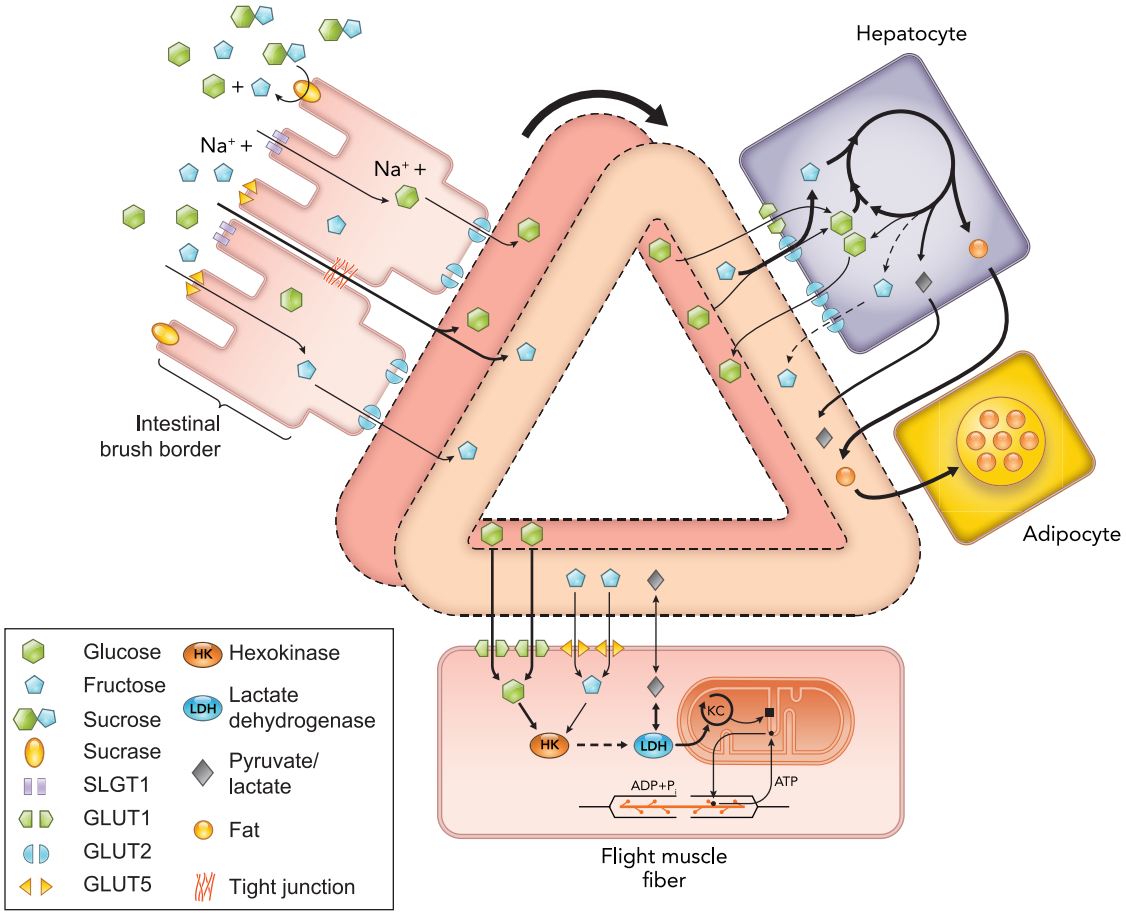

FIGURE 3. **A schematic depicting key aspects of known or hypothesized pathways for nectar sugar absorption and processing in hummingbirds**
This figure highlights probable or suspected flux of glucose and fructose when an animal is foraging and ingesting both sugars (as monosaccharides and as sucrose). Aspects of nectar sugar digestion (*left*), circulatory transport, hepatic processing (*right*), and uptake and oxidation in flight muscle tissue (*bottom*) are shown. Known or hypothesized routes of glucose and fructose (or their metabolites) transmembrane passage (e.g., through glucose transporters, GLUTs), based on a priori expectations arising from known highly conserved mechanisms from model organisms (e.g., humans or rodents) combined with emerging insights from research on hummingbirds, are specifically highlighted. Details of lactate/pyruvate and fat transmembrane passage are omitted for clarity. Known or hypothesized relative rates of flux of glucose, fructose, and metabolites are indicated by the thickness of arrows. Compared with hummingbirds, nectar bats would principally rely on GLUT4-mediated uptake of glucose into muscle cells. Additional known or hypothesized species-specific differences in transport and metabolic pathways are discussed in the text.

and partitioned when both sugars are ingested, as is always the case for wildly foraging individuals. ■

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