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REVIEW

Sugar flux through the flight muscles of hovering vertebrate nectarivores: a review

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Abstract In most vertebrates, uptake and oxidation of circulating sugars by locomotor muscles rises with increasing exercise intensity. However, uptake rate by muscle plateaus at moderate aerobic exercise intensities and intracellular fuels dominate at oxygen consumption rates of 50 % of maximum or more. Further, uptake and oxidation of circulating fructose by muscle is negligible. In contrast, hummingbirds and nectar bats are capable of fueling expensive hovering flight exclusively, or nearly completely, with dietary sugar. In addition, hummingbirds and nectar bats appear capable of fueling hovering flight completely with fructose. Three crucial steps are believed to be rate limiting to muscle uptake of circulating glucose or fructose in vertebrates: (1) delivery to muscle; (2) transport into muscle through glucose transporter proteins (GLUTs); and (3) phosphorylation of glucose by hexokinase (HK) within the muscle. In this review, we summarize what is known about the functional upregulation of exogenous sugar flux at each of these steps in hummingbirds and nectar bats. High cardiac output, capillary density, and blood sugar levels in hummingbirds and bats enhance sugar delivery to muscles (step 1). Hummingbird and nectar bat flight muscle fibers have relatively small cross-sectional areas and thus relatively high surface areas across which transport can occur (step 2). Maximum HK activities in each species are enough for carbohydrate flux through glycolysis to satisfy 100 % of hovering oxidative demand (step 3). However, qualitative patterns of GLUT expression in the muscle (step 2) raise more questions than they answer regarding sugar transport in hummingbirds and suggest major differences in the regulation of sugar flux compared to nectar bats. Behavioral and physiological similarities among hummingbirds, nectar bats, and other vertebrates suggest enhanced capacities for exogenous fuel use during exercise may be more wide spread than previously appreciated. Further, how the capacity for uptake and phosphorylation of circulating fructose is enhanced remains a tantalizing unknown.

Keywords Glucose · Fructose · Hummingbird · Bat · Fuel use · Sugar homeostasis

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Introduction

Exercise physiologists have long appreciated the variation in capacity for sustained exercise among "aerobic" and "non-aerobic" species. Beginning in the 1980s, Ewald Weibel and colleagues (e.g., Weibel et al. 1981; Weibel 1984) formally characterized a suite of physiological and morphological adaptations in comparatively aerobic groups of terrestrial mammals that enhance capacities for uptake, delivery, and use of environmental O_2. These adaptations occur at multiple steps along oxygen's journey through the "oxygen transport cascade:" from the respiratory system through the cardiovascular and circulatory systems to

mitochondria within exercising muscle fibers. Despite differences in aerobic capacity, most vertebrates show similar capacities for powering aerobic metabolism using extramuscular energy sources, such as circulating or dietary sugars. Though pathways for transport of exogenous metabolic substrates to exercising muscle share some elements in common with those that comprise the "oxygen transport cascade", key regulatory steps differ.

When flying to forage on floral nectar, hummingbirds and nectar bats consume oxygen at some of the highest rates observed during exercise in vertebrates (Suarez 1992, 1998; Voigt and Winter 1999; Welch et al. 2008). Oxygen consumption rates in these animals dwarf those seen in even "aerobic" terrestrial mammals. Oxygen consumption per unit body mass in hover-feeding hummingbirds (summarized in: Suarez 1992; Chai and Dudley 1996; Suarez et al. 2011) and nectar bats (Voigt and Winter 1999; Suarez et al. 2011) are 5–7× and 2.5–3.5× higher, respectively, than in dogs (Roberts et al. 1996) exercising at their maximum oxygen consumption rate (VO_{2max}). Following in the footsteps of Weibel and colleagues (Weibel et al. 1981; Weibel 1984), several groups have identified analogous morphological and physiological adaptations in the "oxygen transport cascade" of hummingbirds and nectar bats that make these high aerobic capacities possible. Such enhancements, some of which are common among flying vertebrates in general, include high lung O_2 diffusing capacities, relatively large hearts and high cardiac output, high hematocrits, high capillary densities, and high mitochondrial volume densities (Suarez 1992, 1998; Maina 2000; Suarez et al. 2011).

Recent evidence demonstrates that, unlike terrestrial mammals, hummingbirds and nectar bats possess the ability to match high aerobic capacities with the rapid flux of dietary sugar through exercising muscles. Thus, unlike in most mammals, there is functional upregulation of both the "oxygen transport cascade" (Weibel et al. 1981; Weibel 1984) and the recently named "sugar oxidation cascade" (Suarez et al. 2011). The nectarivorous diet and high metabolic rates in these animals potentially pose great challenges to the maintenance of energy homeostasis and regulation of fuel use. Improved understanding of the physiology and behavior underlying the rapid sugar flux observed in these animals can reveal what adaptations make it possible as well as what the limits are to such flux. Further, it is not yet clear that convergent patterns of sugar flux in hummingbirds and bats are the result of convergent evolution of each organism's physiological systems. This review considers the various steps that comprise the "sugar oxidation cascade" and recent advances in the understanding the functional upregulation of various steps in hummingbirds and nectar bats compared to terrestrial mammals. We identify remaining mechanistic and regulatory questions regarding sugar use in these animals and consider whether some or all aspects of functional upregulation of the "sugar oxidation cascade" may be found more broadly among other vertebrates.

Sugar flux during exercise

Patterns and capacities in terrestrial vertebrates

Variation in mass-corrected aerobic metabolic rates among more and less "aerobic" species of terrestrial mammals has long been appreciated by exercise scientists and comparative physiologists. For example, dogs can achieve peak rates of oxygen consumption that are more than twice as great as similarly sized goats (Roberts et al. 1996). However, while more aerobic species exhibit greater functional capacity for the transport oxygen from the environment to exercising muscle mitochondria, capacities for absorption, delivery, and uptake of dietary glucose or other sugars are largely similar among most mammals thus far studied (Roberts et al. 1996; Weibel et al. 1996; Hoppeler and Weibel 1998). Generally, rates of exogenous glucose uptake and oxidation by exercising muscle peaks at moderate exercise intensities of only 30–40 % of an animal's VO_{2max} (Hoppeler and Weibel 1998; Jeukendrup and Jentjens 2000; Rose and Richter 2005; Wasserman et al. 2011), accounting for 30 % or less of overall metabolic demand (Weber et al. 1996b; Jentjens et al. 2004). As with the "oxygen transport cascade", the flux of glucose from dietary sources, gluconeogenic tissues (e.g., liver), or from that already in circulation to exercising tissues is regulated at multiple steps (Hoppeler and Weibel 1998; Rose and Richter 2005; Wasserman et al. 2011). The pathway for sugar transport to muscle, termed the "sugar oxidation cascade" (Suarez et al. 2011), shares many steps in common with the "oxygen transport cascade". Yet, while increased capacity at shared transport steps results in overall increased oxygen flux capacity in aerobic terrestrial species, overall flux through the "sugar oxidation cascade" is not enhanced to a comparable degree. Further, with few exceptions (e.g., Burelle et al. 2006), studies indicate capacities for the delivery and use of fructose as an oxidative fuel for muscle work in most vertebrates are even more limited (Jandrain et al. 1993; Adopo et al. 1994; Zierath et al. 1995; Kristiansen et al. 1997; Jeukendrup and Jentjens 2000).

In vertebrates, sustained exercise is predominantly fueled by the oxidation of carbohydrates and lipids. With a few notable exceptions, including those highlighted below, exercise intensity largely determines the relative mixture of carbohydrates and lipids oxidized to meet energy demands (Brooks and Mercier 1994; Weber and Haman 2004; Weber 2011). In general, lipid oxidation dominates at low exercise

Table 1 Calculated rates of sugar uptake by flight muscles of ruby-throated (*A. colubris*), Anna's (*C. anna*), and rufous hummingbirds (*S. rufus*) and nectar bats (*G. soricina*). "Integrated" values are time-averaged rates of uptake calculated using the actual time-energy budgets of hummingbirds during experimental periods. "Hovering" values represent rates of sugar intake necessary to match rates of exogenous sugar oxidation during hovering flight in real time. Comparable estimates of sugar uptake by exercising mouse, rat, pygmy goat, and dog skeletal muscle are provided for comparison

	Ruby-throated hummingbird		Anna's hummingbird	Rufous hummingbird	Long-tongued nectar bat	Mouse	Rat		Pygmy goat	Dog
	Glucose ($N = 6$)	Fructose ($N = 6$)	Sucrose ($N = 3$)	Sucrose ($N = 4$)	Sucrose ($N = 7$)	($N = 8$–12)	($N = 5$–13)		($N = 4$)	($N = 3$)
Body mass (g)	2.99 ± 0.15	2.93 ± 0.11	4.98 ± 0.48	3.71 ± 0.12	10.2 ± 0.1	26 ± 1	201–260		29,300	25,000
Integrated	5.51 ± 0.54	6.14 ± 0.59	2.06 ± 0.54	2.21 ± 0.22		0.54 ± 0.06	0.08 ± 0.01*	0.77 ± 0.09*	0.28 ± 0.04†	0.33 ± 0.02†
Hovering	17.13 ± 1.50	16.52 ± 0.79	12.19 ± 1.65	14.64 ± 0.95	7.83 ± 0.44					
Based on data in	Chen and Welch (2014)		Welch and Suarez (2007)		Welch et al. (2008)	Fueger et al. (2004a)	Ploug et al. (1987)		Weibel et al. (1996)	

All data (except body mass) are mean ± SE in μmol g muscle^{-1} min^{-1}

* Isolated soleus muscle subjected to simulated contractions in vitro

† Data are normalized to total skeletal muscle mass (excluding muscles of the head)

intensities. As organisms increase oxygen consumption to meet increased ATP demands at higher exercise intensity, muscle fibers progressively shift to greater relative reliance on carbohydrate oxidation (Brooks and Mercier 1994; Weber and Haman 2004; Weber 2011). This shift in fuel mixture oxidized as a function of exercise intensity coincides with a shift in the reliance on extra- versus intracellular fuel stores. Specifically, the relative reliance on oxidation of circulating lipids and carbohydrates declines above moderate exercise intensities, while the oxidation of lipids and, most importantly, carbohydrates derived from intracellular stores rises (Roberts et al. 1996; Weber et al. 1996a, b; Weber 2011). Oxidation of intramuscular glycogen dominates at the highest exercise intensities, and it is relatively greater glycogen stores and maximal glycogen oxidation rates that, in large part, enable "aerobic" species to achieve greater muscle mass-specific oxygen consumption rates (Weibel et al. 1996; Weber et al. 1996b; Hoppeler and Weibel 1998).

Unlike the breakdown of intracellular glycogen, the uptake and oxidation of exogenous or circulating sugars generally peaks at exercise intensities <50 % of VO_{2max} and fuels roughly 25–35 % of overall muscle ATP turnover (Weber et al. 1996b; Hoppeler and Weibel 1998; Rose and Richter 2005). Rates of glucose uptake and oxidation from circulation reported in running dogs and goats studied by Weibel et al. were remarkably similar, peaking at values of approximately 0.3 μmol g muscle^{-1} min^{-1} (Weber et al. 1996a; Table 1).

Regulation of sugar flux

Glucose

The oxidation of circulating sugars by muscle is constrained by flux limitations at multiple steps along the pathway from ingestion and absorption into circulation to uptake and phosphorylation in muscle. Three principle steps in this cascade are believed to exert significant influence over sugar (principally glucose) uptake and oxidation rates by muscle and these are summarized in Fig. 1, with an emphasis on regulation at or near muscle tissue: (1) the appearance of sugar in the blood, capillary perfusion, and sugar delivery to the extracellular fluid surrounding the fibers; (2) transport of sugars across the sarcolemmal membrane via glucose transporter family proteins (GLUTs); and (3) phosphorylation of hexoses within fibers by hexokinase (Rose and Richter 2005; Bertoldo et al. 2006; Wasserman et al. 2011; Richter and Hargreaves 2013). As muscles increase activity, sugar uptake and oxidation increases due to upregulation of flux at each of these steps.

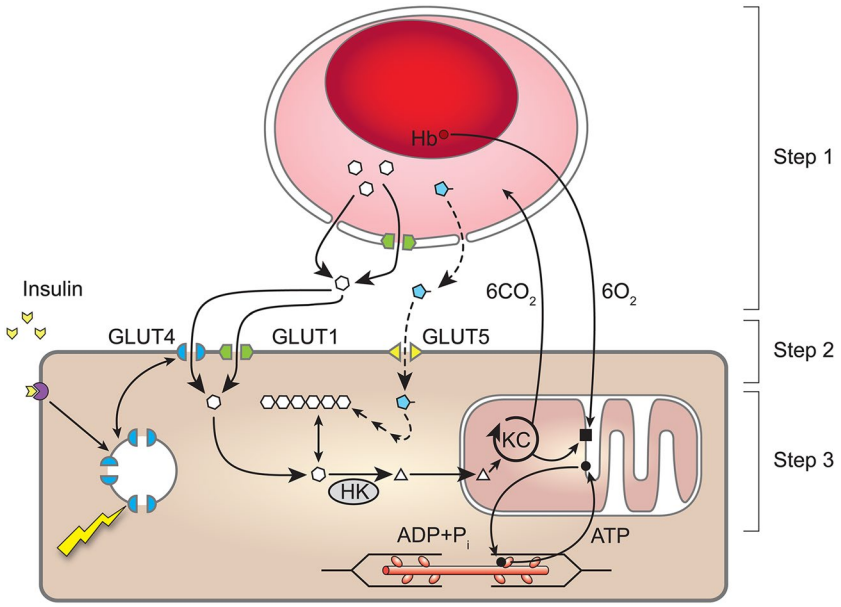

Fig. 1 A model of the flux of circulating sugar into muscle and its regulation in terrestrial mammals. *Step* 1 glucose is delivered to muscle via the circulatory system where it passes to the extracellular space via glucose transporters (GLUTs) and through endothelial junctions in the capillary wall. *Step* 2 glucose uptake across the muscle fiber membrane occurs through the GLUT1 and GLUT4 glucose transporters (Furler et al. 1991; Rose and Richter 2005; Bertoldo et al. 2006). GLUT4-containing vesicles are translocated to the fiber membrane in response to insulin or exercise, promoting uptake capacity. *Step* 3 hexokinase phosphorylates glucose, trapping it in the fiber. Fructose uptake and oxidation occurs at substantially lower rates. See the text for more details. Hexagons indicate glucose, pentagons fructose, chevrons insulin, and triangles acetyl-CoA molecules

Limitations to exogenous sugar uptake and oxidation by exercising muscle begins at the point of absorption into systemic circulation (part of step 1). In humans, maximal rates of absorption, and subsequent oxidation of ingested glucose is generally limited to approximately 1 g/min (Duchman et al. 1997; Jeukendrup et al. 1999; Jeukendrup and Jentjens 2000). By circumventing this limiting step through the direct transfusion of sugar into circulation at greater rates than are observed when sugars are ingested, it is possible to further raise oxidation rates of exogenous sugars during exercise (Hawley et al. 1994) or simulated muscle activity (Ploug et al. 1987). Once in circulation, sugar uptake and oxidation by muscles is constrained by delivery through the circulatory system. During exercise, capacities for sugar delivery by the circulation (step 1) are enhanced primarily by increased capillary perfusion, with capillary density constraining maximum sugar availability to tissues. At the capillary fiber interface, passage of sugars across the capillary walls occurs both through cellular-mediated processes (transport via glucose transporter proteins) as well as via endothelial junctions (Fig. 1; Adamson and Michel 1993; Vock et al. 1996). As a result, the capillary walls are not generally considered a barrier to sugar delivery (Vock et al. 1996). However, sugar delivery to muscles can exert influence on overall uptake oxidation rate. For example, oxidation rates are dependent on supply of glucose to muscle fibers under both in vitro and in vivo conditions as concentrations vary over physiological ranges seen in exercising terrestrial mammals (Nesher et al. 1985; Ploug et al. 1987; Zinker et al. 1993).

By virtue of their transport through the same circulatory system, it is not surprising that some of the regulatory mechanisms that enhance the delivery of oxygen, like capillary hyperemia, also enhance the delivery of sugars. However, while the movement of oxygen from capillaries to muscle fibers occurs via passive diffusion, the movement of sugars into muscle fibers is mediated entirely by members of the glucose transport (GLUT) family of proteins (Mueckler 1994; Uldry and Thorens 2004). This process represents the second major regulatory step (step 2) influencing muscle glucose uptake and oxidation. In terrestrial mammals, facilitated glucose transport into fibers occurs primarily through GLUT1 and, most importantly, GLUT4 (Fig. 1). GLUT1 is constitutively expressed at low levels on the surface of the fiber. When an organism is fasted and at rest, GLUT4 density on the surface of muscle fibers and glucose uptake capacity is relatively low. For this reason, fiber trans-membrane transport of glucose is considered the primary limiting factor to muscle oxidation of circulating or exogenous glucose under such conditions (Post et al. 1961; Furler et al. 1991; Bonadonna et al. 1993; Fueger et al. 2004b; Bertoldo et al. 2006; Fueger et al. 2007).

However, GLUT4 density on the surface of fibers increases dramatically in response to both insulin and fiber activity (exercise). This translocation of GLUT4 from intracellular vesicles to the fiber membrane dramatically enhances glucose transport capacity (Fig. 1; Rose and Richter 2005; Bertoldo et al. 2006; Wasserman et al. 2011; Richter and Hargreaves 2013). Still, even during exercise, the activity of muscle membrane GLUTs is believed to exert significant control over capacities for sugar uptake and oxidation in exercising human and terrestrial mammal muscle fibers, particularly when insulin levels are not simultaneously elevated (Furler et al. 1991; Bonadonna et al. 1993; Rose and Richter 2005; Bertoldo et al. 2006).

Following uptake, glucose is phosphorylated by hexokinase (HK) to glucose-6-phosphate (G6P) which irreversibly traps the monosaccharide within the fiber (Fig. 1; Wasserman et al. 2011). Inhibition of HK activity within muscle cells by relatively high levels of G6P means that this step (step 3) exerts some control over uptake and oxidation of glucose by muscle fibers when at rest and during moderate to intense exercise. At rest, high rates of glycogen synthesis can reduce G6P levels, easing inhibition of HK and enhancing glucose phosphorylation and, by extension, uptake rate (Wasserman et al. 2011). When glycogenolytic pathways are activated during moderate to intense exercise, the resulting production of G6P at high rates from this source similarly limits HK activity (Wasserman et al. 2011).

Fructose

Overall rates of exogenous sugar oxidation during exercise are increased when subjects consume glucose and fructose mixtures compared to isocaloric glucose solutions. This has largely to do with the fact that total rates of sugar absorption across the intestinal wall are greater with glucose–fructose solutions owing to the existence of separate transport mechanisms for each sugar (Jeukendrup and Jentjens 2000). Nevertheless, uptake and oxidation rates of exogenous or circulating fructose in exercising muscle are significantly less than for glucose. Limitations to fructose oxidation in muscle, relative to glucose, occur at each of the three steps outlined above. First, it is generally believed that, in humans at least, capacities for the absorption of fructose across the intestinal brush border (step 1) are lower than for glucose (Jeukendrup and Jentjens 2000). Sugars taken up from the small intestine are delivered into the hepatic portal vein. Unlike most tissues in the body, hepatocytes express GLUT5 at high levels. GLUT5 displays high affinity for fructose and the abundance of this transporter in liver ensures that the majority of fructose is taken up before it can be delivered to muscle (Topping and Mayes 1971; Tappy et al. 1986; Mayes 1993; Lê and Tappy 2006). Unlike most other tissues, hepatocytes express fructokinase, a ketohexokinase with high affinity for fructose. The high activity of fructokinase ensures rapid phosphorylation of fructose to fructose-1-phosphate within hepatocytes (Mayes 1993). Metabolic intermediates derived from fructose-1-phosphate can be converted into lactate or glucose and released back into circulation or incorporated into fatty acids. It is the oxidation of the splanchnic fructose metabolites glucose and lactate which likely accounts for much of the apparent oxidation of exogenous fructose in muscles (Mayes 1993; Jeukendrup and Jentjens 2000; Lê and Tappy 2006). The kinetics of exogenous fructose oxidation in muscle are slower than for glucose because conversion and release of splanchnic fructose metabolites occurs relatively slowly compared to direct circulatory delivery (Jeukendrup and Jentjens 2000). As a result of its effective sequestration in liver tissue, ingestion of fructose typically results in much lower circulating fructose concentrations than for glucose (Fig. 1; Jeukendrup and Jentjens 2000; Lê and Tappy 2006). Thus, delivery of fructose itself to muscle tissue through the circulation (step 1) is comparatively limited.

Even when fructose is directly infused into the circulation at concentrations above those normally seen, rates of fructose uptake and oxidation in muscle are comparatively lower than for glucose (Zierath et al. 1995; Kristiansen et al. 1997). In mammals, the primary GLUTs responsible for glucose uptake into muscle (GLUT1 and GLUT4) exhibit relatively low affinity for fructose (Mueckler 1994; Uldry and Thorens 2004). Instead, transport of fructose into the sarcoplasm primarily occurs through GLUT5, which is present at relatively low density on the sarcolemma of most mammals (Fig. 1; Zierath et al. 1995; Kristiansen et al. 1997). GLUT5 activity, and thus fructose uptake, appears to be responsive neither to insulin nor fiber activity, and is not substantially upregulated during exercise (Zierath et al. 1995; Kristiansen et al. 1997). As a result, overall fructose transport into muscle tissue (step 2) during exercise is comparatively limited.

The rate of phosphorylation of fructose and subsequent oxidation in muscle (step 3) is, like its flux through the other steps, relatively limited compared to glucose. Unlike in liver, there is no fructokinase identified in mammalian muscle tissue and phosphorylation of fructose by hexokinase occurs at relatively low rates, particularly in the presence of glucose (Fig. 1; Mayes 1993). In addition, a substantial proportion of fructose taken up by muscle is used in glycogenesis and, thus, overall fructose metabolism in muscle is depressed during exercise, when glycogenesis is down-regulated (Zierath et al. 1995).

Sugar flux in hovering hummingbirds and nectar bats

Unlike terrestrial mammals, hummingbirds and nectar bats are capable of fueling as much as 100 % of hovering metabolism via the oxidation of sugars ingested only minutes before (Fig. 2; Welch et al. 2006; Welch and Suarez 2007; Welch et al. 2008; Chen and Welch 2014). In these animals, there is concerted upregulation of both the "oxygen" and "sugar transport cascade" (Suarez and Welch 2009; Suarez et al. 2011).

Like other vertebrates, hummingbirds and nectar bats appear to rely predominantly on the oxidation of lipids or carbohydrates to fuel exercise and foraging under most conditions (Welch et al. 2007; Welch et al. 2008). However, in stark contrast to other vertebrates, there appears to be no direct connection between exercise intensity and the mixture of fuels oxidized in these small hovering vertebrates. Instead, the reliance on oxidation of lipids versus carbohydrates appears to be a function solely of dietary status (Suarez et al. 2011). Hummingbirds and nectar bats fuel energetically intensive hovering flight exclusively or predominantly with endogenous lipids at the beginning of the foraging period and following a fast. Just as remarkably, these animals switch to fueling the same energetically intensive hovering exclusively or predominantly with carbohydrate when foraging on nectar. CO_2 derived from the oxidation of labeled, ingested sugars appears in the exhaled breath of hummingbirds and nectar bats just a few minutes after feeding (Fig. 2a). The proportion of exhaled CO_2 derived from exogenous (ingested) sugars rises over the next 30–50 min before plateauing at values near or equal to 100 % (Fig. 2A; Welch et al. 2006; Welch and Suarez 2007; Welch et al. 2008; Chen and Welch 2014). Tracking of isotopically enriched exhaled CO_2, in combination with mask respirometry, has also revealed that turnover of ingested sugars in the pool of actively oxidized substrates is extremely rapid. Specifically, during continuing foraging, the isotopic signature of ingested carbohydrates completely disappears ~20–40 min after ingestion (i.e., declines towards zero; Fig 1B; Welch and Suarez 2007; Chen and Welch 2014). Though disappearance rates have not been measured, it seems likely that the turnover of dietary carbohydrate through nectar bat flight muscle is approximately as rapid. The comparatively high metabolic rate associated with hovering, extensive reliance on newly ingested sugar as a metabolic fuel, and rapid turnover of this exogenous carbon source as a fuel suggests that time-averaged rates of sugar uptake and oxidation by hummingbird and nectar bat flight muscle are dramatically higher than those observed in terrestrial mammals.

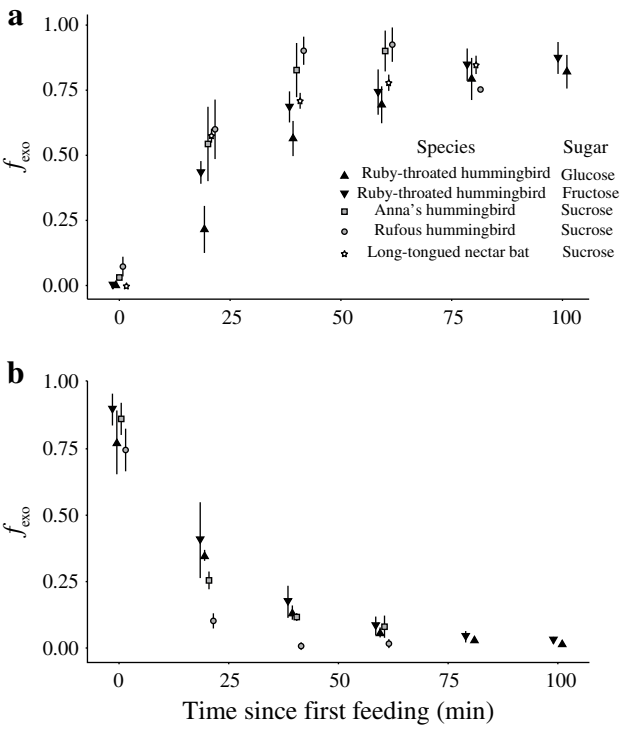

Fig. 2 The proportion of exhaled CO_2 derived from oxidation of exogenous (ingested) sugars (f_{exo}) revealed through stable isotopic tracking as a function of time since first feeding on a given sugar solution in ruby-throated (*A. colubris*), Anna's (*C. anna*), and rufous hummingbirds (*S. rufus*), and nectar bats (*G. soricina*). In *panel* **a**, fasted animals were offered a labeled sugar solution and values rapidly rose towards 1, indicating primary or exclusive reliance on the exogenous sugar as a fuel for flight. In *panel* **b**, an otherwise identical sugar solution with an isotopically distinct signature was offered. Values rapidly declined towards zero indicating rapid turnover of sugar in the pool of actively metabolized substrates and a shift to oxidation of the most recently ingested sugar only. Data are redrawn from (*C. anna* and *S. rufus*: Welch and Suarez 2007; *G. soricina*: Welch et al. 2008; *A. colubris*: Chen and Welch 2014)

Sugar uptake and oxidation by hummingbirds and nectar bat flight muscle

Given the homogenous fast-twitch, highly oxidative fiber type composition (Hermanson et al. 1998; Welch and Altshuler 2009), large relative mass, and high energy turnover of the primary flight muscles of hummingbirds and bats, whole-body measures of oxygen consumption and carbon dioxide production in these animals reveal substantial information about the turnover of carbon in these specific tissues. Specifically, oxygen consumption in hummingbird and nectar bat flight muscle tissue is believed to account for 90 % of whole animal oxygen consumption during hovering (Taylor 1987; Suarez 1992; Welch and Suarez 2007). We also conclude that, because of their small size and high metabolic rates, measurements of respiratory exchange

ratios (RERs) measured in vivo closely approximate cellular respiratory quotients (RQs) in flight muscle tissue (Suarez et al. 2011). Thus, by combining stable isotope tracking-derived measures of the proportion of metabolism supported by oxidation of exogenous sugars with known stoichiometries of carbohydrate oxidation and mitochondrial oxidative phosphorylation (Brand 2005), we can estimate rates of exogenous sugar oxidation in flight muscle tissue.

The ability of hummingbirds and nectar bats to fuel hovering flight completely, or almost completely, with sugar ingested tens of minutes before suggests that calculated exogenous sugar oxidation rates during hovering flight are matched, in real time, by rates of sugar uptake into muscle fibers. This possibility is further supported by the fact that hummingbirds and nectar bats can sustain periods of flight for long durations. Lasiewski famously noted that a juvenile Costa's hummingbird (*Calypte costae*) hovered continuously for ~50 min inside a bell jar (Lasiewski 1963). Estimates of the rates of sugar uptake into hummingbird and nectar bat flight muscle necessary to continuously support hovering flight are shown in Table 1. Muscle mass-specific rates of sugar uptake sufficient to support observed rates of exogenous sugar oxidation range from 7.83 ± 0.44 μmol g^{-1} min^{-1} in nectar bats to 14.64 ± 0.95 μmol g^{-1} min^{-1} in rufous hummingbirds when each was offered sucrose solutions. These values are ~10–19× as high as the values measured in insulin and contraction-stimulated rat soleus muscle measured in vitro (Ploug et al. 1987), ~14–32× the uptake measured in exercising mouse soleus (Fueger et al. 2004a), and ~25–55× the uptake rates in exercising goats and dogs reported in the landmark studies by Weibel et al. (Table 1; Weber et al. 1996a). The slightly smaller ruby-throated hummingbirds exhibited even higher rates of exogenous sugar oxidation and uptake during hovering. When offered glucose solutions, flux of sugar through the flight muscles of ruby-throated hummingbirds was calculated to be 17.13 ± 1.50 μmol g^{-1} min^{-1}. Astonishingly, the flux rate was just as high when fructose was offered (Table 1; 16.52 ± 0.79 μmol g^{-1} min^{-1}). A study by Voigt and Speakman (2007) also suggests that nectar bats can fuel activity with recently ingested fructose. However, because exhaled breath samples were collected immediately after bouts of flight, rather than during, estimation of fructose oxidation rates are not possible (Voigt and Speakman 2007).

Hummingbirds and nectar bats typically alternate periods of flight with periods of perching. Intramuscular glycogen could serve as the primary or sole fuel during periods of flight lasting up to several minutes (Suarez et al. 1990) and glycogen stores could subsequently be rebuilt during periods of perching when sugar oxidation rates might only then fall below uptake rates. Nonetheless, most or all of the CO_2 exhaled by fed hummingbirds and nectar bats is derived from sugar ingested only tens of minutes before (Fig. 2). This rapid turnover of sugar within the pool of actively metabolized substrates indicates that sugar uptake rates into muscle must match or exceed average rates of oxidation over such time periods. Because the half-life of a carbon atom in an ingested sugar molecule within the pool of actively metabolized substrates is approximately 20 min or less (i.e., the fractional rate of disappearance is 0.05 or more; Welch and Suarez 2007; Chen and Welch 2014) sugar uptake rates must match oxidation rates when averaged over a similar time frame.

RER values and the stable isotopic signature of exhaled breath during hover-feeding were found to be similar in both perching and hovering birds (Chen and Welch 2014) and Voigt and Speakman (2007) report stable isotopic signatures from restrained nectar bats which follow similar patterns as those directly measured during hovering (Welch et al. 2008). Thus, it is clear that patterns of exogenous fuel use are not dependent on behavioral state, but are instead a function of nectar availability. Published or measured rates of oxygen consumption and carbon dioxide production were used, in combination with stable isotope tracking-derived estimates of the proportion of metabolism supported by oxidation of exogenous sugar as a function of time since feeding began, to calculate whole animal sugar oxidation rates during perching. Based on consensus values in the literature, we conservatively estimate that carbon turnover in flight muscle accounts for 20 % of whole-body sugar oxidation during perching (Zurlo et al. 1990; Rolfe and Brown 1997). Thus, we were able to estimate rates of exogenous sugar oxidation by flight muscle tissue across both behavioral states in hummingbirds during foraging periods. In combination with time budgets that were obtained during these experiments, we integrated rates of exogenous sugar oxidation over time, and thus estimated minimum necessary time-averaged rates of sugar uptake by flight muscle. We cannot account for possible excess post-exercise oxygen consumption (EPOC) in hummingbirds perching immediately after a hovering bout, and do not know to what extent exogenous glucose or fructose oxidation contribute to excess fuel oxidation during these brief periods. However, by ignoring such possible fuel use, we derive a conservative estimate of time-averaged fuel use in freely behaving hummingbirds. Time-averaged sugar uptake and oxidation rate calculations were not possible for data from nectar bats, because time budgets were not obtained at the time experiments were performed.

Averaged over 20-min time periods, rufous and Anna's hummingbirds fed sucrose solutions took up and oxidized exogenous sugars at 2.21 ± 0.22 and 2.06 ± 0.54 μmol g^{-1} min^{-1}, respectively, in their flight muscles (Table 1;

Welch and Suarez 2007). Time-averaged rates of uptake and oxidation of sugar by ruby-throated hummingbirds flight muscle were higher, averaging 5.51 ± 0.54 and 6.14 ± 0.59 μmol g^{-1} min^{-1} when offered glucose or fructose, respectively (Table 1). These minimum time-averaged rates of exogenous sugar uptake into hummingbird flight muscle are 2.5–8× as great as maximal uptake rates measured in contraction and insulin-stimulated rat soleus muscle in vitro (Table 1; Ploug et al. 1987) and ~4–10× the rate of uptake measured in mouse soleus muscle during exercise (Fueger et al. 2004a), far exceeding any known rates in terrestrial mammals.

Functional enhancement of the "sugar oxidation cascade"

As discussed previously, limitations to the flux and oxidation of dietary glucose and fructose into and through circulation to exercising muscles in terrestrial mammals exist at multiple points. Thus, enhancement of hexose flux through the "sugar oxidation cascade" in hummingbirds and nectar bats necessarily involves functional enhancement at multiple steps. Many of these enhancements, either known or hypothesized, focus on regulatory steps at the capillary–fiber interface or within muscle tissue, and are shown in Fig. 3.

Step 1: Absorption and delivery to muscle tissue

The fact that hummingbirds and nectar bats repeatedly and regularly forage on floral nectar throughout their active periods cannot be overlooked. This behavioral pattern ensures a regular and consistent supply of dietary sugar to the sites of absorption into the body. This constancy of sugar passage through the gut is particularly enhanced by the metering of nectar release from the crop of hummingbirds (Diamond et al. 1986; Karasov et al. 1986).

Once ingested, the absorption of simple sugars from the lumen of the small intestine is enhanced in hummingbirds and nectar bats both by high sucrase activity and by reliance on paracellular absorption of lumenal contents. Given their sucrose-rich diet, high sucrase activity in hummingbirds and nectar bats ensures that virtually all ingested sucrose is hydrolyzed to glucose and fructose such that absorption is possible (Hernandez and Martinez del Rio 1992; Schondube and Martinez del Rio 2004). Similarly, reliance on paracellular absorption of sugars in addition to the cellular-mediated pathways for absorption enhances sugar uptake by hummingbirds and nectar bats (McWhorter et al. 2006; Caviedes-Vidal et al. 2007). This enhanced functional capacity for sugar uptake across the intestinal brush border improves the potential for uptake of both glucose and fructose equally and circumvents a limitation experienced by terrestrial mammals when ingesting higher sugar loads (Jeukendrup and Jentjens 2000).

Pathways for the delivery of oxygen and sugar to muscle cells converge in the circulatory system. Thus, most of the adaptations in circulatory function that enhance oxygen delivery in hummingbirds and nectar bats also enhance sugar delivery. As with oxygen, the rate at which sugar is delivered to a given volume of tissue is a function of the amount of the substrate carried per unit volume of blood, and the relative tissue perfusion rate. Convective transport of sugar and oxygen is enhanced by relatively greater heart mass and high rates of cardiac output (Johansen 1987; Bishop 1997). High capillary density and capillary-to-fiber contact area ratios in hummingbirds (Fig. 4) and, generally in bats, ensure comparatively greater capacities for delivery of sugars to the extracellular spaces surrounding fibers (Mathieu-Costello et al. 1992a, b).

Importantly, blood glucose levels in recently fed hummingbirds and nectar bats spike at concentrations that are much higher than those observed in comparatively sized terrestrial mammals (~40 and ~25 mM, respectively; Beuchat and Chong 1998; Braun and Sweazea 2008; Kelm et al. 2011). These levels dwarf those observed even in fed nectarivorous amethyst sunbirds (*Chalcomitra amethystina*; ~16 mM; Witteveen et al. 2014) or fruit bats (~8 mM; Mqokeli and Downs 2012). Interestingly, fasted blood glucose levels in hummingbirds (~17 mM) remain much higher than in fasted mammals of comparable size (Beuchat and Chong 1998), while blood glucose levels in fasted nectar bats fall to much lower levels (~5 mM, depending on activity level; Kelm et al. 2011). The methods used to determine blood glucose levels in previous studies (e.g., oxidation by glucose oxidase) are highly specific for glucose and not fructose (Wilson and Turner 1992). Thus, unfortunately, circulating fructose levels in fasted and fed hummingbirds and nectar bats remain unknown. While little fructose escapes uptake into the liver to enter systemic circulation in humans and most mammals (Mayes 1993; Lê and Tappy 2006), it is not certain that, given the high dietary intake, high circulating glucose concentrations, and apparent rapid oxidation of fructose during exercise (Voigt and Speakman 2007; Chen and Welch 2014), fructose is destined for a similar fate in hummingbirds and nectar bats. Instead, circulating fructose levels may be comparable to circulating glucose levels in both hummingbirds and nectar bats (Fig. 3).

Step 2: Transport across the sarcolemmal membrane

As in other animals (Adamson and Michel 1993; Vock et al. 1996), passive diffusion via endothelial junctions likely permits high rates of fructose delivery to extracellular fluid even if specific fructose transporters (e.g., GLUT5) are not found in abundance on capillary endothelium (Fig. 3). However, transport of sugars into muscle cells is believed to occur exclusively via GLUT proteins. Given the

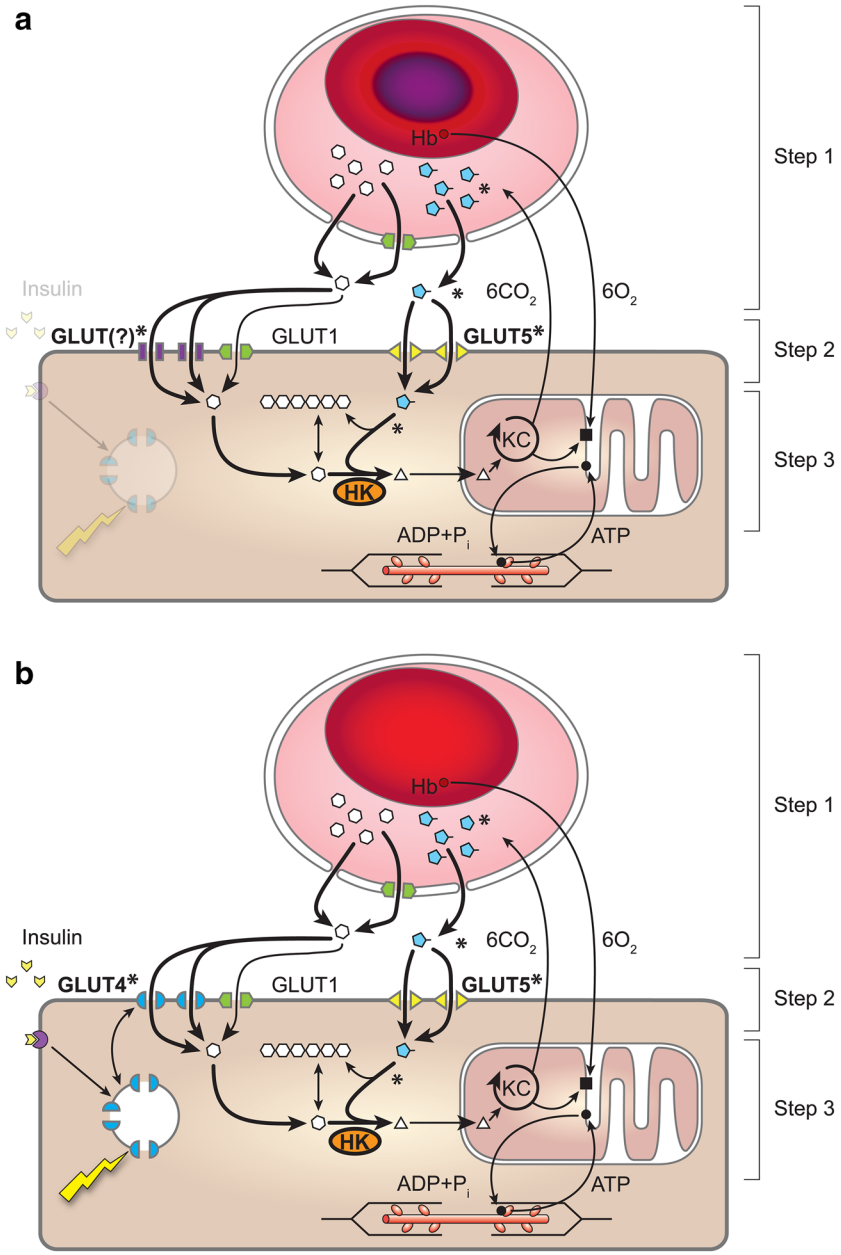

Fig. 3 Contrasting models of pathways for circulating (exogenous) sugar uptake by skeletal muscle in hummingbirds (**a**), and nectar bats (**b**). Model elements with known or suspected functional enhancements compared to terrestrial mammals are in *bold*. *Asterisks* indicate elements where the mechanism for enhanced functional capacity is hypothetical or poorly understood. In mammals, muscle contractile activity and insulin signaling independently, or in combination, enhance sugar uptake by promoting translocation of GLUT4 from intracellular vesicles to the fiber surface. Low transport capacity through GLUT5 and low affinity of hexokinase II (HK) for fructose largely limit the ability of mammalian muscle fibers to use this sugar directly as a fuel during exercise. *Step* 1 high levels of circulating sugars and high capillary density enhance delivery to tissues in both hummingbirds and nectar bats (**a**, **b**). *Step* 2 increased capacity for sugar transport across the sarcolemmal membrane promotes availability of sugars for use in glycolysis. In bats, enhanced uptake capacity is likely conferred through high abundance of GLUT4 transporters (**b**). GLUT4 is not found in hummingbirds and they are presumably, like other birds, insulin insensitive (**a**). It is not yet known what differences in GLUT expression or activity confer elevated capacities for glucose uptake. *Step* 3 High hexokinase (HK) activity enables high rates of glucose phosphorylation and entry into glycolysis in both groups (**a**, **b**). Evidence from in vivo studies suggest that hummingbird and nectar bat muscle fibers may take up and oxidize fructose at rates comparable to glucose (Voigt and Speakman 2007; Chen and Welch 2014). The mechanisms enhancing fructose flux through steps 2 and 3 are not well understood in either group. Enhanced GLUT5 activity may confer high rates of fructose uptake (*step* 2 **a**, **b**). The mechanism for rapid phosphorylation of fructose and incorporation into glycolysis (*step* 3) is unknown. Hexagons indicate glucose, pentagons fructose, chevrons insulin, and triangles acetyl-CoA molecules

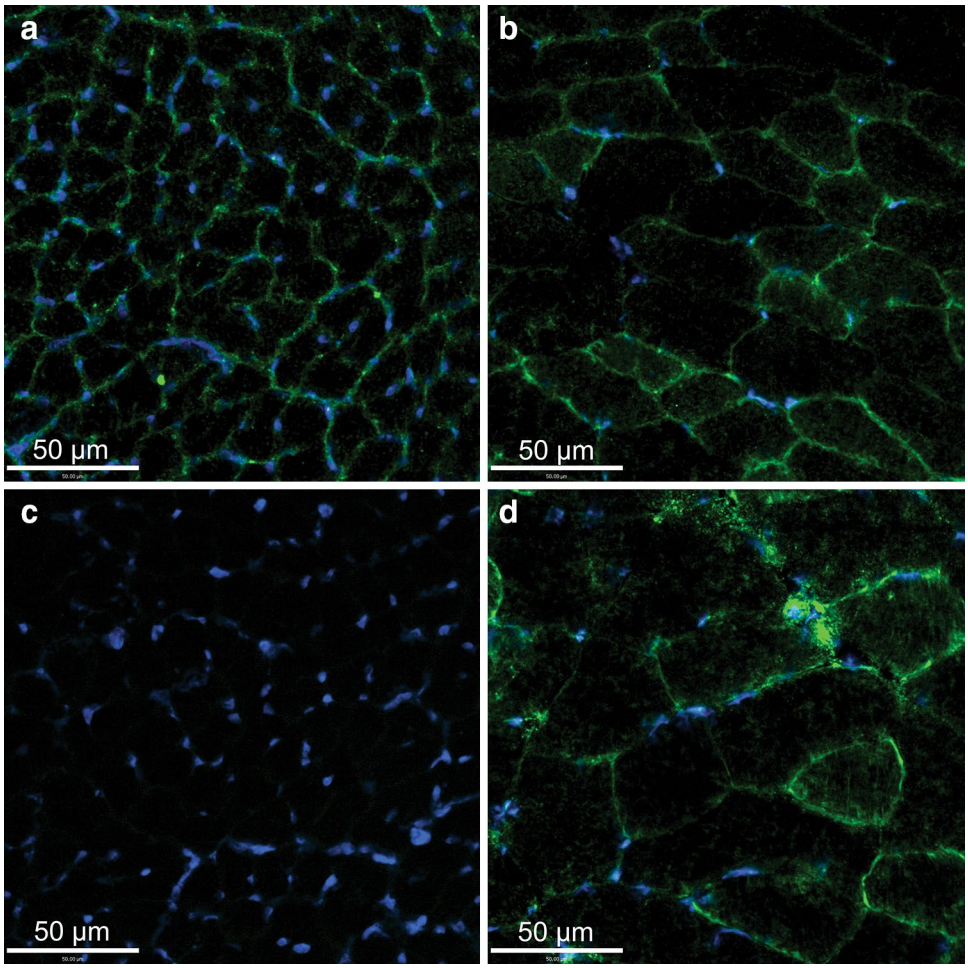

Fig. 4 Glucose transporter (GLUT) staining in the pectoralis of the ruby-throated hummingbird (*A. colubris*; **a**, **c**) and mouse gastrocnemius (*Mus musculus*; **b**, **d**). Staining for GLUT1 (**a**, **b**) is apparent in both species whereas, staining for insulin/exercise-responsive GLUT4 (**c**, **d**) appears only in the mouse. Staining for GLUT1, which is localized to muscle fiber membranes and capillary endothelia, also highlights the marked difference in fiber size, capillary density, and thus capillary-to-fiber surface ratios between the two species. Nuclei were visualized in each tissue by counterstaining with DAPI. Figure from Welch et al. (2013)

importance of this transport step in defining overall capacities for the oxidation of exogenous sugars during exercise in terrestrial mammals and regulation of fuel use and blood glucose homeostasis, it seems likely that this step is also quite important in defining and regulating flux through the "sugar oxidation cascade" in hummingbirds and nectar bats. Unfortunately, relatively little is known about the tissue distribution and abundance or transport kinetics of specific GLUT proteins or how their interspecific variation might enable the observed high rates of sugar flux into hummingbird and nectar bat flight muscles. However, what little information exists suggests that substantial differences likely exist regarding the mechanisms that regulate sugar uptake in birds versus bats generally, and hummingbirds versus nectar bats specifically.

In terrestrial mammals, insulin and exercise-responsive GLUT4 plays the primary role in glucose uptake into muscle fibers (Rose and Richter 2005; Bertoldo et al. 2006; Wasserman et al. 2011). A recent study demonstrated that flight activity is key to the regulation or lowering of blood glucose levels in nectar bats following feeding and the authors suggest that this is due to exercise-induced (and, likely, insulin-induced) upregulation of GLUT4 activity on the surface of active muscle fibers (Fig 3B; Kelm et al. 2011). This suggests that mechanisms for sugar uptake into flight muscle and regulation of blood sugar in nectar bats are qualitatively similar to the highly conserved pathways found in terrestrial mammals. However, what differences underlie the substantially greater capacity for sugar uptake in nectar bats compared to terrestrial mammals remains unclear. One recent study has examined the evolution of the gene encoding the GLUT4 transporter in several bat species, including the nectar bat, *Glossophaga soricina* (Shen et al. 2012). However, no clear signature of selection on the putative GLUT4 sequence was observed in New World species, including *G. soricina*. Thus, clear differences in GLUT4 protein sequence that might confer enhanced transport activity are not obvious. Preliminary, unpublished evidence suggest relatively abundant levels of the GLUT4 protein in nectar bat flight muscle (R. Lee-Young, D. Wasserman, R. Suarez, unpublished). However, these data do not yet prove that a high density of GLUT4 on nectar bat flight muscle fiber membranes fully, or even partially, account for increased capacities for glucose uptake. In

terrestrial mammals, GLUT4 exhibits very low affinity for fructose (Mueckler 1994; Uldry and Thorens 2004). Instead, it is GLUT5, present at low levels on skeletal muscle membranes, that provides the limited fructose transport capacity that exists (Zierath et al. 1995; Kristiansen et al. 1997). It is possible that comparatively higher GLUT5 expression in nectar bat flight muscle underlies high rates of fructose uptake (Fig. 3b). However, since expression levels for most GLUT proteins in nectar bat muscle are not known, this cannot be confirmed.

While nectar bats may be able to sustain high rates of glucose uptake into flight muscle tissue through a high density of GLUT4 transporters, this cannot be true for hummingbirds. Hummingbirds do not transcribe GLUT4 mRNA or express a GLUT4 protein homolog in any tissue thus far examined (Fig. 3 A, Fig. 4; Welch et al. 2013). Hummingbirds are not alone in failing to express GLUT4, as it seems the gene for this protein does not reside within any avian genome (Sweazea and Braun 2006; Welch et al. 2013; but see Diamond and Carruthers 1993; Thomas-Delloye et al. 1999). The lack of an insulin-responsive GLUT4 protein homolog helps explain why birds are relatively insulin-unresponsive and exhibit relatively high blood glucose levels even when fasted (Fig 3A: Braun and Sweazea 2008; Polakof et al. 2011). However, these findings only raise further questions about the mechanisms underlying increased capacities for sugar uptake in hummingbird flight muscle. Welch et al. (2013) noted that GLUT1 protein staining was present in hummingbird flight muscle tissue (Fig. 4), though the relative abundance of this protein could not be determined since antibody-binding efficiency was unknown. In addition, this group noted the presence of GLUT3 mRNA in hummingbird pectoralis, but could not examine protein expression because no suitable commercial antibody was available (Welch et al. 2013). Pathways for fructose uptake into hummingbird skeletal muscle are even less well understood. In the absence of information, there is no a priori reason to hypothesize that GLUT1 or GLUT3 exhibit high affinity or capacity for fructose transport in birds. Instead, it may be that GLUT5, or another, possibly undescribed GLUT transporter, exhibits both high affinity and activity for fructose and is present in sufficient density to account for the high apparent rates of fructose uptake (Fig 3a). For now, this remains only a tantalizing area for further study.

Step 3: Phosphorylation of hexoses within muscle fibers

A series of studies by Suarez and colleagues have measured flight muscle mass-specific maximal activities HK in nectar bats and hummingbirds that are roughly 7–8× those measured in rat soleus (Blomstrand et al. 1983; Suarez et al. 2009). V_{max} values for HK measured in the nectar bat and rufous hummingbird pectoralis muscles are greater than the calculated required rates of flux of glucose through glycolysis necessary to fully and continuously fuel hovering flight via oxidative phosphorylation (Suarez et al. 2009) and are sufficient to fully explain the differences in apparent capacities for flux of glucose through flight muscle in these animals compared to rats. HK activity is considered a major rate-limiting step to the flux of glucose through glycolysis and oxidative phosphorylation in mitochondria. Available evidence indicates that maximal activities of HK and other glycolytic and oxidative enzymes are dramatically higher in hummingbirds generally (Fernandez et al. 2011). Thus, differences in metabolic biochemical capacities for glucose flux, as well as high mitochondrial volume densities in hummingbird and nectar bat flight muscles, seem sufficient to fully explain the dramatically enhanced flux of exogenous glucose through these tissues that are observed in vivo (Fig 3; Welch and Suarez 2007; Welch et al. 2008; Chen and Welch 2014).

Capacities for the phosphorylation of fructose within hummingbird and nectar bat flight muscles, and the entry of this sugar directly or indirectly into glycolysis, have not been explicitly measured. It is tempting to consider the possibility that a specific fructokinase, like that found in hepatocytes, is abundantly expressed in the flight muscle tissue of nectar bats and hummingbirds (Fig. 3). Only additional research can confirm if such capacities for the direct phosphorylation of fructose exist within the skeletal muscles of these species and identify the biochemical pathways that potentially permit rapid incorporation of fructose into glycolysis.

Evolution of the "sugar oxidation cascade" and open questions

In exploiting similar dietary niches, hummingbirds and nectar bats have converged with respect to many physiological, morphological, and behavioral traits, supporting high rates of aerobic metabolism and sugar oxidation in muscle in both groups. Many of the functional adaptations which enhance the uptake and delivery of oxygen to exercising muscle in hummingbirds and nectar bats are present in other vertebrates. For example, because flight is a comparatively energetically demanding form of locomotion, birds and bats varying widely in mass all exhibit greater functional respiratory and cardiovascular capacities than similarly sized terrestrial vertebrates (Maina 2000; Bishop 2005). Similarly, many birds and bats display functional enhancement of aspects of at least some steps in the "sugar oxidation cascade". Hummingbirds and nectar bats are not the only vertebrate nectarivores. Further, many frugivores feed regularly and repeatedly on foods which are also rich in simple sugars. Thus, the consistent availability of plentiful dietary sugar, which is an ecological and behavioral

prerequisite for predominant reliance on exogenous sugars as a fuel for exercise, is common to many more groups than hummingbirds and nectar bats. At the very least, it is certain that all nectarivore and frugivore specialists face challenges to the maintenance of blood sugar homeostasis that are not seen in comparatively better studied groups of vertebrates.

Other vertebrates share some of the additional physiological traits that, in hummingbirds and nectar bats, contribute to enhancement of the "sugar oxidation cascade". Many flying vertebrates exhibit pronounced paracellular absorption of nutrients, including sugars, across the intestinal wall (Caviedes-Vidal et al. 2007). Thus, they would presumably possess enhanced capacities for sugar absorption compared to terrestrial vertebrates which must rely solely on cellular-mediated uptake. Indeed, many old world sunbirds and honeyeaters display dietary sugar assimilation capacities comparable to hummingbirds and nectar bats (reviewed in Nicolson and Fleming 2014). Some, though not all, nectar and fruit-feeding bats and birds also exhibit significantly greater intestinal sucrase activity compared to other species (Martínez del Rio 1990; Hernandez and Martinez del Rio 1992; Jackson et al. 1998; Napier et al. 2013; Nicolson and Fleming 2014). Birds generally exhibit blood sugar levels that are 2–3 times higher than those of terrestrial vertebrates of similar mass (Braun and Sweazea 2008) and, in some frugivorous and nectarivorous bats and birds, blood sugar levels rise as a result of feeding, though not as dramatically as has been observed in hummingbirds and nectar bats (Kelm et al. 2011; Mqokeli and Downs 2012; Witteveen et al. 2014). Taken together, these data suggest that the availability of sugar to active muscle tissue in many flying vertebrates may be sufficient to enable the support of muscle activity primarily, or completely, with exogenous sugar. Evidence suggests this is the case for in Egyptian fruit bat (*Rousettus aegyptiacus*; Amitai et al. 2010). Unfortunately, frustratingly few studies have empirically examined the ability of other frugivorous or nectarivorous birds, bats, or terrestrial vertebrates to fuel exercise predominantly or completely with exogenous sugars. Even less evidence exists regarding the ability of any vertebrates other than hummingbirds and nectar bats to support exercise metabolism via the oxidation of exogenous (or endogenous) fructose.

Existing data clearly indicate that, at least in some respects, the mechanisms that enable high rates of sugar uptake into muscle fibers, and thus contribute to the regulation of blood sugar homeostasis, are quite different in hummingbirds and nectar bats (Fig. 3). Bats may achieve high rates of sugar flux and regulation of blood sugar levels via enhancement of an otherwise typically mammalian physiology based, in part, on insulin and exercise-sensitive regulation of GLUT4 function. Yet, hummingbirds, like all birds, lack this transporter and blood sugar levels in birds do not respond strongly to insulin. Nectar bats and hummingbirds have converged with respect to patterns of exogenous fuel use and flux despite diverging with respect to aspect of sugar transport and regulatory physiology. So, it seems that evolution has produced at least two mechanistic solutions among vertebrates for the common problem of fueling an energetically demanding form of locomotion while subsisting on a diet rich in simple sugars.

Summary

A growing body of evidence indicates that certain small, nectarivorous vertebrates possess capacities for the transport and oxidation of dietary sugar which far exceed those common to most mammals, and thought to be highly evolutionarily conserved. Specializations of dietary ecology, behavior, and physiology all contribute to the observed phenomenal capacities for the use of sugar in hummingbirds and nectar bats. The physiological differences which underlie increased capacities for flux through the "sugar oxidation cascade" occur in multiple systems and tissues and impact flux through each of the three traditionally emphasized regulatory steps: (1) absorption and delivery of sugar to muscle tissues; (2) transport across the sarcolemmal membrane; and (3) intracellular phosphorylation of hexose. Yet, while many of the functional enhancements that make up the "sugar oxidation cascade" are increasingly well understood, important gaps in our understanding remain. The mechanisms which enhance sugar transport across the fiber membrane (step 2) are clearly fundamentally different among hummingbirds and nectar bats (and among mammals and birds, generally). And mechanisms which underlie the suggested ability of hummingbird and nectar bat flight muscles to uptake and oxidize fructose directly remain unknown.

Diets that are rich in simple sugars, as well as at least some of the physiological traits that support high rates of exogenous sugar flux through exercising muscle in hummingbirds and nectar bats, are common to several groups of mammals and birds. Yet, only through additional research will it be possible to conclude if the unusual patterns of fuel use observed in hummingbirds and nectar bats are also more common than previously appreciated. At the very least, continuing work on capacities for the flux of both glucose and fructose through the "sugar oxidation cascade" in hummingbirds, nectar bats, and other vertebrate groups promises to reshape our understanding of fuel use during exercise and sugar homeostasis among all vertebrates.

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