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The sugar oxidation cascade: aerial refueling in hummingbirds and nectar bats

Raul K. Suarez[1,*], L. Gerardo Herrera M.[2] and Kenneth C. Welch, Jr[3]

[1]Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106, USA, [2]Estación de Biología de Chamela, Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 21, San Patricio, Jalisco 48980, México and [3]Department of Biological Sciences, University of Toronto, Scarborough, Ontario M1C 1A4, Canada

*Author for correspondence (suarez@lifesci.ucsb.edu)

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Summary

Most hummingbirds and some species of nectar bats hover while feeding on floral nectar. While doing so, they achieve some of the highest mass-specific $\dot{V}_{O_2}$ values among vertebrates. This is made possible by enhanced functional capacities of various elements of the 'O_2 transport cascade', the pathway of O_2 from the external environment to muscle mitochondria. Fasted hummingbirds and nectar bats fly with respiratory quotients (RQs; $\dot{V}_{CO_2}/\dot{V}_{O_2}$) of ~0.7, indicating that fat fuels flight in the fasted state. During repeated hover-feeding on dietary sugar, RQ values progressively climb to ~1.0, indicating a shift from fat to carbohydrate oxidation. Stable carbon isotope experiments reveal that recently ingested sugar directly fuels ~80 and 95% of energy metabolism in hover-feeding nectar bats and hummingbirds, respectively. We name the pathway of carbon flux from flowers, through digestive and cardiovascular systems, muscle membranes and into mitochondria the 'sugar oxidation cascade'. O_2 and sugar oxidation cascades operate in parallel and converge in muscle mitochondria. Foraging behavior that favours the oxidation of dietary sugar avoids the inefficiency of synthesizing fat from sugar and breaking down fat to fuel foraging. Sugar oxidation yields a higher P/O ratio (ATP made per O atom consumed) than fat oxidation, thus requiring lower hovering $\dot{V}_{O_2}$ per unit mass. We propose that dietary sugar is a premium fuel for flight in nectarivorous, flying animals.

Key words: energetics, fuel use, muscle, metabolism, convergent evolution, foraging, thermoregulation, mitochondria.

Introduction

Metabolic fuel use during exercise is a subject of great interest to human exercise physiologists, comparative biochemists and ecological physiologists. With few known exceptions, e.g. tsetse flies (Bursell and Slack, 1976), a pattern that has emerged is that energy metabolism during exercise relies primarily on carbohydrate or fat as substrates. Another pattern, based on studies of humans and several other vertebrate species, is that as exercise intensity increases and the maximum rate of O_2 consumption ($\dot{V}_{O_2max}$) is approached, the fractional contribution of fatty acid oxidation to ATP production declines whereas that of carbohydrate oxidation increases (Brooks, 1998; Weber and Haman, 2004). As animals approach $\dot{V}_{O_2max}$ during exercise, muscle glycogenolysis accounts for an increasing fraction of the carbohydrate used (Weber et al., 1996). The greater reliance on glycogen than on blood glucose in animals exercising close to $\dot{V}_{O_2max}$ appears to be due to limitations to glucose phosphorylation in muscle fibers (Fueger et al., 2004). However, the capacity to fuel exercise metabolism directly from dietary sources is also limited. For example, in humans, only ~30%, at most, of exercise metabolism can be directly fueled by ingested sugar (Jentjens et al., 2004). In this paper, we describe remarkable cases of deviation from the above paradigm, made possible through the operation of a pathway we name the 'sugar oxidation cascade'.

The sugar oxidation cascade is the path of carbon from flowers, through digestive and cardiovascular systems, across capillary walls, muscle cell membranes and into the mitochondria in hovering, nectarivorous vertebrates (Fig. 1). The operation of this pathway enables hummingbirds and nectar bats to engage in aerial refueling, i.e. to use recently ingested sugar to directly fuel their exercising muscles during hovering flight. In many respects, the sugar oxidation cascade is analogous to the 'oxygen transport cascade' (Weibel, 1984; Weibel et al., 1981), which is the path of O_2 from the external environment, through the respiratory and cardiovascular systems and into the mitochondria of exercising muscles. The two cascades share some common elements, operate in parallel and ultimately converge in the mitochondria of the flight muscles (Fig. 1). Although we shall focus on our own work using hummingbirds (primarily rufous hummingbirds, *Selasphorus rufus* Gmelin 1788) and, more recently, Pallas' long-tongued bats (*Glossophaga soricina* Pallas 1766; hereafter nectar bats), we shall also draw on the findings of others to describe the elements of the cascade and the biological context in which it operates.

Brief history and ecological context

While foraging for free coffee in the 1970s, Peter Hochachka and his graduate students pondered a metabolic mystery: when hummingbirds hover to forage on floral nectar, they ingest mainly sugar. Do they immediately synthesize fat from the ingested sugar, only to break it down shortly after to fuel foraging flight? At this time, the remarkable ability of birds to build up fat stores and to use it to fuel energy metabolism was already well appreciated (Blem, 1976). Although hummingbirds, like many other species of birds, were known to undergo premigratory fattening and to use fat to fuel migratory flight (Odum et al., 1961), it seemed implausible, even to naïve biochemists, that they would use fat to fuel foraging activity.

Much has been learned concerning hummingbird biology in the decades that followed. Rufous hummingbirds spend about half of their lives migrating between breeding grounds in the Pacific Northwest and overwintering habitats in Mexico (Calder, 1987). As fat stores are depleted, they stop along their migration route to refuel. Hummingbirds obtain most of their dietary calories from

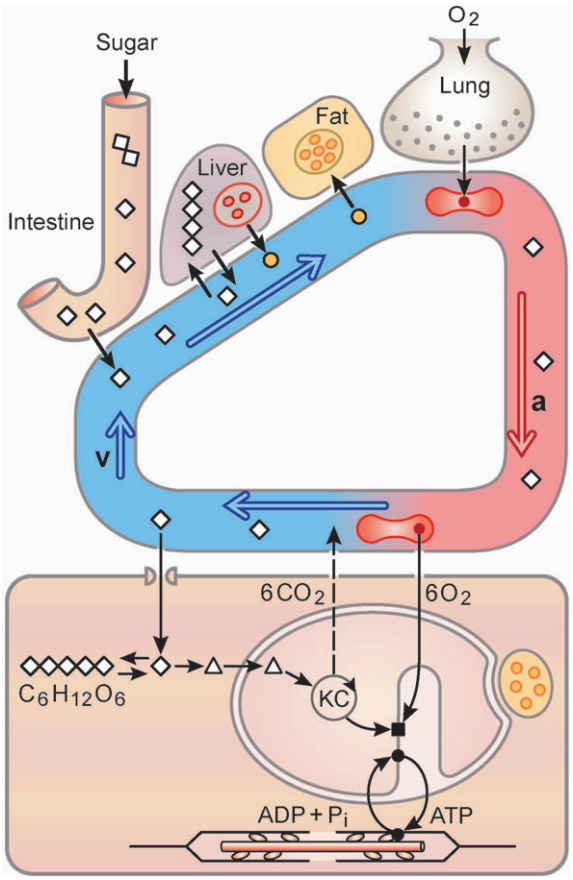

Fig. 1. Sugar oxidation and O_2 transport cascades in hover-feeding hummingbirds (*Selasphorus rufus*) and nectar bats (*Glossophaga soricina*). During steady-state aerobic exercise, red blood cells load O_2 at the lungs, travel in the arterial system (red) and unload in muscle capillaries. Deoxygenated blood (blue) travels back to the lungs. O_2 unloaded from red blood cells diffuses into muscle mitochondria, which account for ≥90% of whole-body $\dot{V}_{O_2}$. In parallel, ingested sucrose (double diamond), glucose and fructose (single diamonds) cross the intestinal epithelium and enter the circulation as monosaccharide (single diamonds). Some fraction of the blood monosaccharide pool is taken up by the liver and converted to glycogen (string of diamonds) and fat (circles). Another fraction is taken up by exercising flight muscles by GLUT-mediated transport. Monosaccharide taken up is metabolized to glycogen or broken down (triangles) and oxidized, yielding CO_2 in the Krebs cycle (KC) and H_2O in the cytochrome *c* oxidase reaction, the last step in the electron transport chain (black square). For simplicity, ATP turnover is represented by hydrolysis at myofibrils and resynthesis at the mitochondrial inner membrane. The diagram illustrates how the sugar oxidation and O_2 transport cascades share common elements, operate in parallel and converge in muscle mitochondria. Redrawn and modified from Weibel et al. (Weibel et al., 1996).

floral nectar (Powers and Nagy, 1988). They visit flowers that produce nectars rich in sucrose (Baker et al., 1998) and prefer sucrose solutions to solutions of glucose + fructose (Martinez del Rio, 1990). Fat synthesis from ingested sugar occurs at rates that allow the birds to gain up to 10% of body mass per day (Carpenter et al., 1983). Rufous hummingbirds tend to fly for short durations while foraging on floral nectar (Diamond et al., 1986). Foraging often occurs in subalpine meadows where, in the early morning hours, ambient temperature (T_a) can be near freezing (C. L. Gass, personal communication) (Gass et al., 1999). At low T_a, thermogenic mechanisms are activated (Bicudo et al., 2002), resulting in elevation of rates of energy expenditure (Lasiewski, 1963; Lopez-Calleja and Bozinovic, 1995; Lotz et al., 2003). To maintain energy balance or to achieve net energy gain and synthesize fat under these conditions, the birds increase foraging activity (Gass et al., 1999; Suarez and Gass, 2002). Low air densities encountered at high altitude further raise the energetic costs incurred during foraging (Welch and Suarez, 2008). Thus, hummingbird foraging often occurs under environmental conditions easily characterized as 'extreme'.

In contrast with hummingbird-visited flowers, those visited by nectarivorous bats produce nectars with low sucrose and high glucose + fructose concentrations (Baker et al., 1998). However, the nectar bats that we recently studied do not discriminate between solutions of sucrose and glucose + fructose of equal energy content (Rodriguez-Pena et al., 2007). *Glossophaga soricina* does not migrate, unlike some other nectarivorous bat species (Fleming et al., 1993; Morales-Garza et al., 2007), and does not accumulate much body fat (McNab, 1976). However, they are capable of energetically expensive hovering (Voigt and Winter, 1999; Winter et al., 1998), allowing mechanistic comparison of a nectarivorous hovering mammal with hummingbirds.

Fuel oxidation

Hummingbirds in flight sustain some of the highest mass-specific rates of aerobic metabolism ($\dot{V}_{O_2}/M_b$, where M_b is body mass) known amongst vertebrates (Suarez, 1992). Nectar bats display lower but, nevertheless, impressive hovering $\dot{V}_{O_2}/M_b$ values (Voigt and Winter, 1999; Winter et al., 1998), similar to the maximal rates observed in shrews exposed to low T_a (Fons and Sicart, 1976). Respiratory physiologists have found that the flux of O_2 is regulated at multiple steps in the oxygen transport cascade (di Prampero, 1985; Jones, 1998; Wagner, 1996). It is probably because of such distributed regulation (i.e. the absence of a single, rate-limiting step) that enhanced functional capacities at multiple steps in the pathway of O_2 have evolved in hummingbirds and bats. These have been the subject of previous reviews (Maina, 2000; Suarez, 1992; Suarez, 1998) and include high lung O_2 diffusing capacities, large hearts and high heart rates, high cardiac outputs, high haematocrits, high muscle capillary densities, high mitochondrial volume and cristae surface densities.

Both hummingbirds and nectar bats possess flight muscles consisting exclusively of fast-twitch, oxidative fibers (Grinyer and George, 1969; Hermanson et al., 1998; Suarez et al., 1991). It is likely that, when in flight, 90% or more of their whole-body $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (rate of CO_2 production) values are accounted for by flight muscles (Suarez, 1992; Taylor, 1987). Given their small body masses and high $\dot{V}_{O_2}/M_b$ values during flight, whole-body O_2 and CO_2 fluxes measured by respirometry yield respiratory exchange ratios ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) that are likely to approximate cellular respiratory quotients (RQs). These data can be used to determine the nature of metabolic fuel(s) oxidized and to estimate ATP turnover rates, given what is known concerning the stoichiometries of carbohydrate and fatty acid oxidation, as well as mitochondrial oxidative phosphorylation (Brand, 2005).

We have used mask respirometry, essentially as previously described (Bartholomew and Lighton, 1986), to measure $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ of hummingbirds and nectar bats as they hover to feed on sugar solutions dispensed within the mask. When these animals begin to forage in the fasted state, RQ values are close to 0.7, indicating that fatty acids are oxidized to provide most of the energy for flight (Suarez et al., 1990; Welch et al., 2008) (Fig. 2). As further feeding

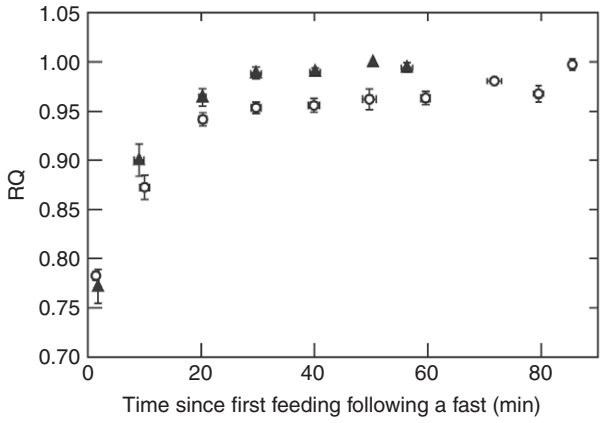

Fig. 2. Respiratory quotients (RQ values) during hover-feeding as a function of time after fasting in rufous hummingbirds (*Selasphorus rufus*; triangles) and nectar bats (*Glossophaga soricina*; circles). In these experiments, hummingbird foraging bouts were typically brief because, after each visit to the feeder, the birds usually return to the perch before hover-feeding again. Nectar bats tended to fly more continuously and perch intermittently under the conditions of our experiments, so data obtained from bats represent repeat visits to the feeder with irregularly spaced perching sessions. During the first feeding bout, fasted animals displayed RQ values close to 0.7, indicating that hovering is fueled mainly by fatty acid oxidation. RQ values progressively increased to ~1.0 as animals fed on sucrose solutions, indicating a transition from fatty acid to carbohydrate oxidation. During repeated foraging on sucrose solutions, RQ values remained close to 1.0. Data are means ± s.e.m. Redrawn from Welch et al. (Welch et al., 2008).

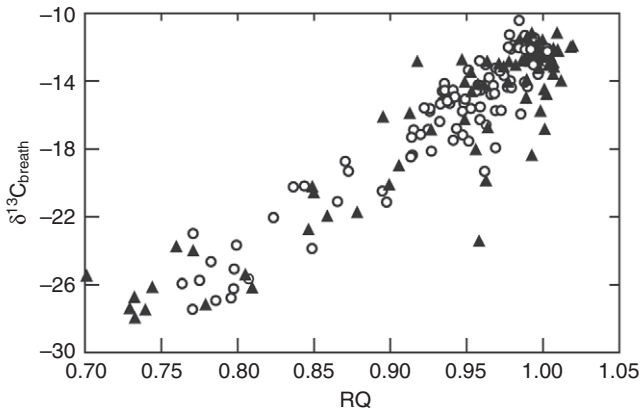

Fig. 3. $\delta^{13}C$ values of expired CO_2 as a function of respiratory quotients (RQ values) in hover-feeding rufous hummingbirds (*Selasphorus rufus*; triangles) and nectar bats (*Glossophaga soricina*; circles). More negative $\delta^{13}C$ values when RQ values are close to 0.7 indicate oxidation of fatty acids previously synthesized from beet sugar provided in the diet. $\delta^{13}C$ values increase as RQs approach 1.0 and cane sugar provided in the experiment becomes the main fuel used for flight. Data points represent individual measurements. Breath samples were taken under the same experimental conditions as described in the Fig. 2 legend. Redrawn from Welch et al. (Welch et al., 2008).

occurs, RQ values progressively increase to ~1.0. This indicates that, in both hummingbirds and nectar bats, carbohydrate progressively takes over as the main fuel for oxidative metabolism in the flight muscles as they hover to feed on sucrose. The data shown in Fig. 2 were obtained from rufous hummingbirds and nectar bats; similar results have been obtained from Anna's (*Calypte anna*) (Welch et al., 2008) and broadtailed (*Selasphorus platycercus*) (Welch et al., 2006) hummingbirds.

Further insights into fuel use during foraging come from the use of carbon stable isotopes (Welch et al., 2006; Welch et al., 2008; Welch and Suarez, 2007). These experiments take advantage of the lower $^{13}C/^{12}C$ ratio of sucrose synthesized by C3 photosynthesis in beets compared with that of sucrose from sugar cane, which is made by C4 photosynthesis. This results in a more negative (i.e. more ^{13}C-depleted) $\delta^{13}C$ of beet sugar than cane sugar, where:

$$\delta^{13}C = \frac{[^{13}C]/[^{12}C]}{R_{std}} - 1 , \quad (1)$$

and $R_{std}=[^{13}C]/[^{12}C]$ of marine limestone from the Pee Dee Cretaceous belemnite in South Carolina or an artificial version from Vienna (see McNevin et al., 2007). Hummingbirds and nectar bats maintained on diets enriched in beet sugar expire CO_2 with $\delta^{13}C$ values similar to that of beet sugar. In the fasted state, initial foraging flights yield RQ values close to 0.7, indicating fatty acid oxidation. However, the fatty acids oxidized in these animals were previously synthesized from beet sugar, so $\delta^{13}C$ values of CO_2 expired during hovering are low. The animals were then provided cane sugar *via* the feeder in the mask. Repeated feeding bouts were accompanied by progressively increasing $\delta^{13}C$ values of expired CO_2. Fig. 3 shows that the $\delta^{13}C$ values increase with RQ, indicating that the direct oxidation of recently ingested cane sugar accounts for an increasing fraction of the energy required for flight as the animals transition from oxidizing fat to oxidizing carbohydrate. When the animals are repeatedly foraging and consistently display RQ values close to 1.0, dietary sugar oxidation provides ~95 and 80% of the energy required for flight in hummingbirds and nectar bats, respectively (Fig. 4). Reliance of oxidative metabolism primarily on recently ingested sugar was also previously shown in nectar bats (Voigt and Speakman, 2007) using breath samples from animals restrained shortly after exercise. By contrast, ingested sugar can fuel only ~30%, at most, of human energy expenditure during exercise (Jentjens et al., 2004).

Elements of the sugar oxidation cascade

The operation of the sugar oxidation cascade is analogous to the aerial refueling performed by certain types of high-performance aircraft in that the ingested fuel is quickly used by oxidative reactions that convert the energy stored in organic compounds into mechanical work and heat. In common with such aircraft, nectarivorous, flying vertebrates require high capacities for the delivery of O_2 and fuel to their flight motors. Evidence for this is seen at the level of the digestive system: enhanced digestive capacities are made possible by high intestinal sucrase activities (Hernandez and Martinez del Rio, 1992; Schondube et al., 2001) as well as the combined use of active and passive mechanisms (involving paracellular movement) for sugar movement across the intestinal epithelium (McWhorter et al., 2006). The use of both active and passive mechanisms by fruit bats (Caviedes-Vidal et al., 2008) suggests that both mechanisms occur in nectar bats as well. Hummingbird cardiac outputs are estimated at approximately 5 times body mass per minute (Johansen, 1987), ensuring high capacities for convective transport of blood-borne fuels, whereas high capillary to muscle fiber surface area ratios (Mathieu-Costello et al., 1992; Suarez et al., 1991) would enhance diffusive capacities, not just for O_2 and CO_2, but for metabolic fuels as well.

Upon entry into exercising muscle fibers, glucose is phosphorylated to glucose 6-phosphate (G6P). Nectar bat and

hummingbird flight muscles possess extraordinarily high capacities for glucose phosphorylation to G6P, catalyzed by the enzyme hexokinase (Suarez et al., 1990; Suarez et al., 2009). These biochemical capacities are estimated by measurement of enzyme maximum velocity (V_{max}) values (where $V_{max}=k_{cat}\times[E]$, k_{cat} is catalytic efficiency and [E] is enzyme concentration); these establish the upper limits to physiological flux rates (Newsholme and Crabtree, 1986; Suarez, 1996). Given high hexokinase V_{max} values, it seems likely that flight muscle membranes in hummingbirds and nectar bats would also possess high capacities for glucose transport, made possible by high levels of glucose transporter (GLUT) expression. No information is yet available concerning hummingbird GLUTs. However, in sparrow (*Passer domesticus*) skeletal muscles, Sweazea and Braun report the presence of mRNA coding for GLUT1 and GLUT3 and the absence of GLUT4 (Sweazea and Braun, 2006). In addition, they show immunohistochemical evidence of protein expression of GLUT1 and GLUT3, as well as western blots showing the absence of GLUT4. Similarly, Seki et al. report the absence of GLUT4 in broiler chickens (Seki et al., 2003). Sweazea and Braun argue that previous reports of GLUT4 in avian skeletal muscles are probably erroneous (Sweazea and Braun, 2006). On the basis of the established role of GLUT4 in exercise and insulin-stimulated glucose transport in mammalian skeletal muscles (Huang and Czech, 2007), nectar bats would be expected to possess high levels of GLUT4 protein expression in their flight muscles. This appears to be the case, based on preliminary results (R. Lee-Young, D. Wasserman and R.S., unpublished).

In exercising hummingbird and nectar bat muscle fibers, abundant mitochondria operate as O_2 sinks and intracellular gradients drive diffusive O_2 fluxes from capillary red blood cells to mitochondrial cytochrome *c* oxidase. The O_2 and sugar oxidation cascades converge at the level of flight muscle mitochondria, where the oxidation of each mole of $C_6H_{12}O_6$, requiring 6 mol O_2, leads to the production of 6 mol CO_2 by decarboxylation reactions in the Krebs cycle and 6 mol H_2O by the cytochrome *c* oxidase reaction, as well as 2.41 mol ATP per O atom consumed (Brand, 2005), assuming operation of the malate–aspartate shuttle for cytoplasmic redox balance (Suarez et al., 1986; Suarez et al., 1990). It appears that the evolution of high capacities for flux through the O_2 transport cascade, required because of small body size and high mass-specific power output during flight, has partly set the stage for high capacities for carbon flux through the sugar oxidation cascade as a consequence of multiple shared elements.

In vivo flux through the sugar oxidation cascade

According to Chantler: "The most noble aim of the biochemist, often discussed when inebriate, seldom when sober, is to relate the *in vitro* to the *in vivo*" (Chantler, 1982). It is therefore of interest to consider the rate at which the sugar oxidation cascade might operate during hovering flight. To address this, it is useful to first consider the more general question of how energy metabolism is regulated in muscles performing steady-state work. Muscles are biological machines and their mechanical power output largely determines their rates of ATP hydrolysis. In locomotory muscles performing repeated cycles of contraction and relaxation during steady-state exercise, the power output of a given volume of muscle is a function of its operating frequency (contraction cycles/time), stress (force/cross sectional area) and strain (fractional change in length per contraction) (Pennycuick and Rezende, 1984). In synchronous muscles, most of the ATP used during exercise is hydrolyzed by actomyosin-ATPase and Ca^{2+}-ATPase (Homsher, 1987; Homsher and Kean, 1978; Szentesi et al., 2001). Metabolic control analysis performed using skinned rat soleus fibers reveals that, among various processes, the greatest degree of control (i.e. the highest control coefficient) over mitochondrial respiration is exerted by ATP hydrolysis (Wisniewski et al., 1995). Given current knowledge concerning the stoichiometries of fuel oxidation and oxidative phosphorylation (Brand, 2005), $\dot{V}_{O_2}$ values during hover-feeding (when RQ=1) can be used to estimate rates of muscle ATP turnover and flux rates through the sugar oxidation cascade (Table 1). Interspecific comparisons reveal that hexokinase operates at much higher fractional velocities in the flight muscles of nectarivorous animals (Suarez et al., 1990; Suarez et al., 2009) than in the locomotory muscles of other species (Suarez et al., 1997). Thus, in rufous hummingbirds and nectar bats, high rates of glucose phosphorylation *in vivo* result from high levels of hexokinase expression as well as the operation of this enzyme at high fractional velocities during foraging flight (Suarez et al., 1990; Suarez et al., 1997; Suarez et al., 2009).

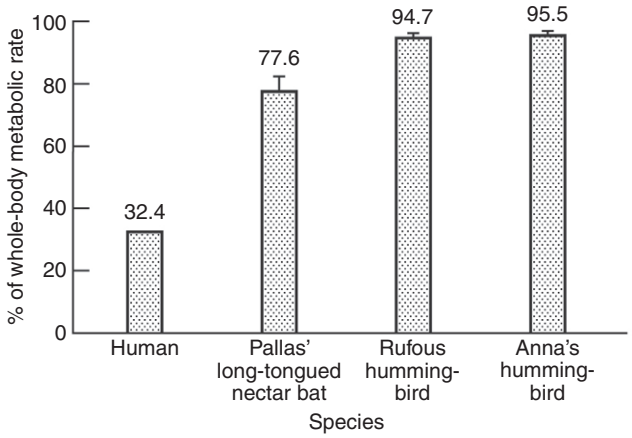

Fig. 4. Percent of whole-body metabolic rate during repeated hover-feeding that is fueled by dietary sugar. Data from two species of hummingbirds (*Selasphorus rufus* and *Calypte anna*) and nectar bats (*Glossophaga soricina*) obtained during hovering are compared with data obtained during bipedal locomotion in humans (Jentjens et al., 2004). Redrawn from Welch et al. (Welch et al., 2008).

The impressive rate at which the sugar oxidation cascade can operate is dramatically illustrated by the results of laboratory experiments simulating the cold mornings encountered by migratory rufous hummingbirds at foraging sites in the late summer (Gass et al., 1999). These experiments were performed in an environment chamber wherein T_a was held at 5°C. Because digestive efficiency in hummingbirds is close to 100% (Diamond et al., 1986; McWhorter and Martinez del Rio, 2000), the energy intake rate is calculated from the volume and concentration of sucrose solution ingested over the 4 h duration of the experiments. Mass gain, mainly in the form of fat, results when energy intake rates exceed rates of energy expenditure. Mass loss indicates that intake rates are insufficient to meet energy requirements and depletion of fat stores results. Maintenance of mass indicates that the time-averaged dietary energy intake rate equals energy expenditure. In these experiments, low T_a combined with low energy content of sucrose solutions dispensed at the feeder elevated energetic costs and drove up foraging activity. Fig. 5 shows that hummingbirds fed various volumes of 15 and 20% sucrose at 5°C tended to lose mass, whereas maintenance of mass or mass gain

Table 1. O_2 consumption ($\dot{V}_{O_2}$), muscle ATP turnover, glucose and palmitate oxidation rates during hovering in rufous hummingbirds (*Selasphorus rufus*) and nectar bats (*Glossophaga soricina*)

	Rufous hummingbird (*N*=6)	Nectar bat (*N*=7)
Whole-body $\dot{V}_{O_2}$ (ml O_2 g^{-1} h^{-1})[a]	33.27±0.63	24.48±0.65
Flight muscle $\dot{V}_{O_2}$ (ml O_2 g^{-1} h^{-1})[b]	119.77±2.25	84.74±2.26
Flight muscle ATP turnover rate (μmol g^{-1} min^{-1})[c]	429.52±8.09	264.26±7.06
Glucose oxidation rate (μmol g^{-1} min^{-1})[d]	14.85±0.28	9.14±0.24
Palmitate oxidation rate (μmol g^{-1} min^{-1})[e]	2.78±0.05	1.97±0.05

[a] $\dot{V}_{O_2}$ data are from previous studies (Suarez et al., 2009; Welch et al., 2007).
[b] Percent of body mass that is flight muscle in hummingbirds is highly variable because of large seasonal and diurnal changes in body fat content. Flight muscle mass was assumed to be 25% of body mass for *S. rufus* (Chai and Millard, 1997; Wells, 1993) and 26% of body mass for *G. soricina* (Dudley and Winter, 2002).
[c] Calculated assuming 2.41 mol ATP per O atom consumed when RQ=1.0 (Brand, 2005).
[d] Calculated assuming 6 mol O_2 are consumed per mole of glucose oxidized using flight muscle $\dot{V}_{O_2}$.
[e] Calculated assuming 32 mol O_2 are consumed per mole of palmitate oxidized using flight muscle $\dot{V}_{O_2}$×1.15 to account for 15% higher $\dot{V}_{O_2}$ when RQ=0.7 compared with RQ=1.0 (Brand, 2005; Welch et al., 2007).
All data are presented as means ± s.e.m.

were possible when birds were provided 30% sucrose. The regression intersects the line, indicating zero mass change at a maximum sustained metabolic rate (Hammond and Diamond, 1997; Peterson et al., 1990) of 1 W. When energy metabolism is completely fueled by dietary sucrose, the time-averaged rate of glucose ingestion and oxidation by a 4 g bird, given an energy content of 16.5 kJ g^{-1} sucrose, is 218 mg h^{-1} (Gass et al., 1999). Time-averaged metabolic rates of ~0.81 W are sustainable for 12 h periods (Beuchat et al., 1979). Thus, at low T_a, rufous hummingbirds achieve the highest known maximum sustained metabolic rates among vertebrates, fueled by the sugar oxidation cascade when dietary energy intake rates equal rates of energy expenditure (Hammond and Diamond, 1997; Peterson et al., 1990).

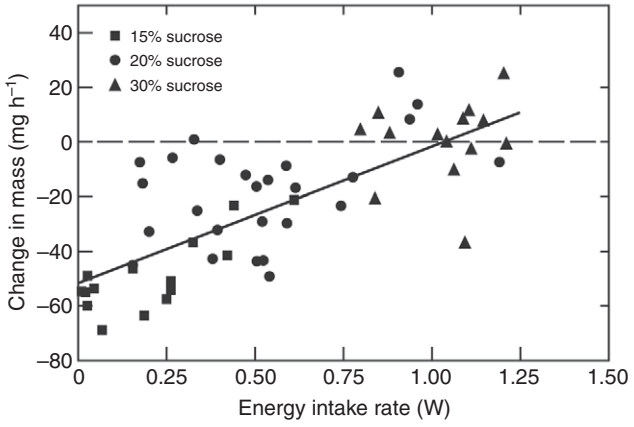

Fig. 5. Change in mass as a function of energy intake rate in watts in rufous hummingbirds (*Selasphorus rufus*) kept for 4 h at 5°C in an environment chamber with one perch and one feeder. The perch was placed on top of an electronic balance, allowing bird mass to be monitored. Perching triggered the release of a given volume and concentration of sucrose solution at the feeder which the birds emptied at each visit. Intake rates were estimated from volume and concentrations of sucrose solutions consumed, assuming 16.5 kJ g^{-1} sucrose and 100% digestive efficiency. The mean mass of birds was 4 g. Energy balance (zero change in mass) occurs at an energy intake rate of 1 W. Birds tended to lose mass when provided 15 and 20% sucrose solutions and were able to stay in energy balance or make an energetic profit when provided 30% sucrose. These data show that the sugar oxidation cascade can support a time-averaged metabolic rate of 250 W kg^{-1}, the highest time-averaged metabolic rate known among vertebrates during which energy intake=energy expenditure. Redrawn from Gass et al. (Gass et al., 1999).

Caveats and open questions

The scientific method requires that we consider our proposal as a working hypothesis that should be questioned and subjected to further test. We have named the proposed process the 'sugar oxidation cascade' in recognition of the differing sugar compositions of the floral nectars ingested by hummingbirds and nectar bats in nature (Baker, 1975; Baker et al., 1998), as well as the lack of information concerning fructose metabolism in these animals. In humans, up to half of ingested fructose is converted by the liver to glucose, which then appears in the blood (Delarue et al., 1993). During exercise at 60% of V_{O_2max}, orally ingested glucose and fructose directly contribute up to 15 and 12%, respectively, to energy production (Adopo et al., 1994). In rat skeletal muscles, maximal rates of fructose transport are approximately eightfold lower than rates of glucose transport (Kristiansen et al., 1997). Upon entry into the sarcoplasm, fructose is phosphorylated and converted to glycogen, lactate or CO_2, but these occur at rates far lower than when glucose is the precursor (Zierath et al., 1995). However, the predominant role of glucose in muscle energy metabolism in humans and rats should not lead to blind extrapolation to nectarivorous animals that ingest fructose – both as a monosaccharide and as part of the sucrose molecule – at much higher mass-specific rates than rats and humans.

An intriguing question concerns the role played by muscle glycogen. Upon entering muscle fibers and after phosphorylation by the hexokinase reaction, glucose can be converted to glycogen or broken down in the glycolytic pathway and oxidized. The flight muscles of hummingbirds and nectar bats are unique among vertebrate skeletal muscles in that they have hexokinase V_{max} values so high that glucose oxidation can completely account for $\dot{V}_{O_2}$ values during flight. Such high capacities for glucose phosphorylation are also observed in the flight muscles of frugivorous but not insectivorous bats (Yacoe et al., 1982). This indicates that sugar-rich diets and not just small body size or flight account for the high capacities for muscle glucose phosphorylation. By contrast, nectarivory is common to all hummingbird species; all hummingbird species we have examined so far display high hexokinase V_{max} values in their flight muscles (M. J. Fernandez and R.S., unpublished). These activities are far higher than in other non-nectarivorous avian species (e.g. Bishop et al., 1995; Blomstrand et al., 1983; Crabtree and Newsholme, 1972) that have flight muscles with hexokinase V_{max} values that are insufficient to allow glucose to serve as the sole oxidative fuel during flight. In possessing high enzymatic capacities as well as in displaying high

physiological rates of glucose phosphorylation, hummingbird and nectar bat flight muscles appear rather similar to vertebrate hearts (Kashiwaya et al., 1994) that oxidize glucose and long-chain fatty acids in proportions that depend upon physiological circumstances (Collins-Nakai et al., 1994). Even while performing steady-state aerobic work, glycogen turnover occurs in hearts; glycogenolysis serves to buffer hexose phosphate concentrations and its contribution to the fueling of aerobic metabolism changes during transitions in work rate (Goodwin et al., 1995; Goodwin et al., 1998). Thus, when considering energy metabolism in the flight muscles of hummingbirds and nectar bats, there is good reason to doubt the validity of the assertion that "…the substrate of muscle glycolysis is glycogen, not glucose, and hexokinase is part of the glycogen synthesis pathway" (Fell, 2000), as well as to turn such doubt into further testable hypotheses.

Ecological and evolutionary implications

Nectarivory and hovering flight were once traits found only among insects. In becoming hovering nectarivores, hummingbirds and nectar bats have converged, evolving enhanced capacities for O_2 flux and sugar oxidation. Foraging behavior and metabolic regulation appear to have coevolved in rufous hummingbirds (Suarez and Gass, 2002; Suarez et al., 1990). Because foraging bouts tend to be brief in the wild (Diamond et al., 1986), such behavior would tend to ensure the oxidation of dietary sugar and to minimize the use of fat as a fuel. This avoids the inefficiency of a futile cycle involving the expenditure of energy to synthesize fat, followed by the breakdown of fat made from sugar to fuel further foraging. Instead, depletion of fat stores while foraging is avoided or minimized and oxidation of recently ingested sugar is favored.

Another advantage derived from the oxidation of sugar is the 15% higher yield of ATP per oxygen atom consumed (the P/O ratio) as compared with the oxidation of long-chain fatty acids (Brand, 2005). This suggests that, to support the energetic requirements of a unit mass of hummingbird during hovering, 15% lower $\dot{V}_{O_2}$ is required when sugar is oxidized as compared with fat. This hypothesis is supported by results showing that $\dot{V}_{O_2}$ declines by ~15% as hummingbirds transition from the fasted state, when flight is fueled by fat, to the fed state, when flight is fueled by sugar (Welch et al., 2007). Many species of hummingbirds forage at relatively high altitude under conditions wherein flight performance might be adversely affected by hypobaric hypoxia (Altshuler and Dudley, 2002). Thus, we hypothesize that ingested sugar serves as a premium fuel for hummingbird flight.

Over the past half century, the field of comparative physiology has generated a fascinating and valuable body of knowledge concerning organismal function, physiological adaptation and patterns of functional variation across species. The exploration of functional biodiversity in the natural world continues to be central to the research agenda of the field. The research that led to the concept of the sugar oxidation cascade exemplifies how natural history, animal behavior and ecology can serve as the inspiration for comparative physiological questions. In turn, research in comparative physiology can lead to new concepts and greater understanding of the nature and extent of biodiversity. The answers to mechanistic, physiological questions continue to enrich the banquet at which ecologists, behaviorists and evolutionary biologists can feast.

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