# Stoking the Brightest Fires of Life Among Vertebrates

**Raul K. Suarez and Kenneth C. Welch**

**Abstract** Hummingbirds and nectarivorous bats in flight display some of the highest rates of aerobic metabolism among vertebrates. Analysis of the pathway of oxygen, i.e., the "oxygen transport cascade", reveals the concerted upregulation of capacities for $O_2$ flux from the external environment, through the respiratory and cardiovascular systems, into muscle mitochondria. Pathways for aerobic energy metabolism are highly conserved, but enzymatic capacities for carbohydrate and fatty acid oxidation, as well as for aerobic ATP synthesis, are also upregulated in concert. Despite evidence indicating sufficient capacities for fatty acid oxidation to support hovering, repeated bouts of hover-feeding in hummingbirds and nectar bats involve the oxidation of carbohydrate. Recent studies reveal that recently ingested sugar directly fuels flight, giving rise to the concept of the "sucrose oxidation cascade". The ecological and bioenergetic advantages conferred by sugar oxidation during foraging are discussed.

## 1 Introduction

Kleiber's "fires of life", referring to the processes involved in aerobic energy metabolism (Kleiber 1961), must rely on a supply of carbon substrates while consuming $O_2$ and producing $CO_2$. It is therefore appropriate that a volume concerning respiratory physiology should include a chapter dedicated to the subject of fuel use. Here, we shall examine aerobic pathways of muscle energy metabolism, how they are fueled, and how they operate during exercise in species that achieve some of the highest known mass-specific metabolic rates among vertebrates.

Small hummingbirds in steady, hovering flight display mass-specific rates of oxygen consumption $(\dot{V}O_2)$ (Bartholomew and Lighton 1986; Lasiewski 1963; Suarez

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**Table 1** Metabolic rates of some animals during exercise

| | Mass (g) | $VO_2$ (ml $O_2$/g × h) | References |
|---|---|---|---|
| Honeybee | 0.078 | 101.0 | Suarez et al. (1996) |
| Rufous hummingbird | 3.4 | 38.3 | Suarez et al. (1990) |
| Nectar bat | 11.7 | 21.6 | Winter et al. (1998) |
| Etruscan shrew | 2.4 | 24.1 | Fons and Sicart (1976) |
| Human athlete | 70,000 | 4.3 | Blomstrand et al. (1986) |

Not intended as a comprehensive list, the purpose of the table is to facilitate comparison of rates sustained during routine hovering in rufous hummingbirds (*Selasphorus rufus*) and nectar bats (*Glossophaga soricina*) with summit metabolic rates of Etruscan shrews (*Suncus etruscus*) at low temperature, human athletes during exercise at $VO_2$max, and honeybees (*Apis mellifera*) in hovering flight. In the case of exercising animals, because >90% of $VO_2$ is accounted for by locomotory muscles, data concerning muscle mass allow the estimation of $VO_2$ per unit muscle mass. When RQ values are known, flux rates through pathways of carbohydrate or fatty acid oxidation and ATP turnover rates can be calculated

et al. 1990) about tenfold higher than those observed in humans engaged in maximal, aerobic exercise (i.e., $VO_2$ max) (Blomstrand et al. 1986) (Table 1). During molting, or in response to mass-gain (Epting 1980), or when exposed to low-density, normoxic air (Chai and Dudley 1995), hummingbirds are able to increase their hovering metabolic rates further by about 50%, thereby achieving the highest known mass-specific, aerobic metabolic rates among vertebrates. Nectarivorous bats during hovering flight display mass-specific $VO_2$ values (Voigt and Winter 1999; Welch et al. 2008; Winter et al. 1998) about 40–50% lower than hummingbirds but are, nevertheless, impressive when compared with even smaller mammals, e.g., similar to summit metabolic rates measured in shrews exposed to low ambient temperature (Fons and Sicart 1976). To burn most brightly in these animals, the fires of life require the highest flux rates of $O_2$ and $CO_2$, and rates of fuel catabolism known among vertebrates. During high-intensity, aerobic exercise, >90% of whole-body metabolic rate is accounted for by locomotory muscles (Suarez 1992; Taylor 1987). This greatly facilitates mechanistic studies concerning the relationships between biochemical flux capacities and actual (physiological) flux rates. This is especially true of hummingbirds, in which two sets of flight muscles, the pectoralis and supracoracoideus, consist exclusively of fast-twitch, oxidative fibers (Grinyer and George 1969; Lasiewski et al. 1965; Mathieu-Costello et al. 1992; Rosser and George 1986). These features allow the use of whole-body $VO_2$ and $VCO_2$ values for the estimation of rates of oxidation of carbon substrates in a single cell-type (Suarez et al. 1990).

## 2 Pathways of Fuel Oxidation

Among the major achievements of twentieth century biology was the elucidation of the major pathways involved in the oxidative metabolism of carbohydrates, fats and amino acids. Comparative biochemists and physiologists then built upon this

foundation, exploring natural variation on the themes established by Meyerhof, Warburg, Krebs, and their successors. This body of work has revealed that pathways for the catabolism of various substrates occur as modular units. These modules are found in various combinations in homologous cell types across species. In locomotory muscles, broad patterns of quantitative variation in enzymatic flux capacities across species are now well-documented. The stage is now set for studies of functional significance in the context of both ecology and evolution, the primary focus of this chapter.

In evolving to become small and to hover while feeding on floral nectar, hummingbirds and nectarivorous bats have both invaded a niche previously occupied exclusively by insects (Dudley 2000). The flight muscles of hummingbirds (Suarez et al. 1986, 1990) and nectarivorous bats (Suarez et al., unpublished) possess high enzymatic flux capacities for the oxidation of carbohydrates and long chain fatty acids. They use the same, highly conserved metabolic modules found in all vertebrate endotherm red muscles and hearts (Fig. 1). These include the

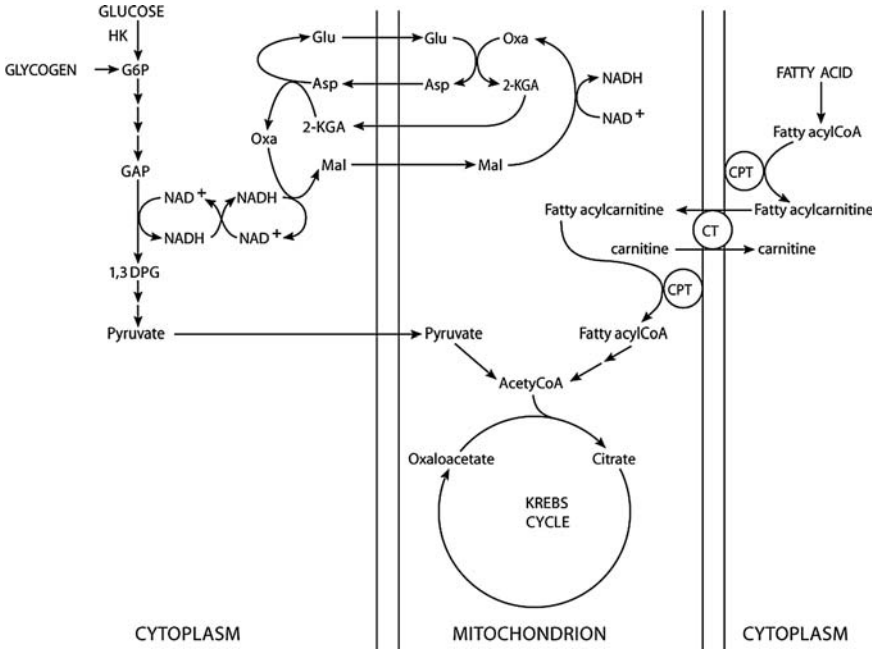


**Fig. 1** Pathways of carbohydrate and fatty acid oxidation in hummingbird flight muscles. A number of the modules referred to in the text are apparent: glycolysis, the malate–aspartate shuttle (*both on the left*), fatty acid oxidation (*on the right*), the Krebs cycle (*bottom*). Metabolic pathways are highly simplified and are redrawn from Suarez et al. (1990). Abbreviations: *HK*; hexokinase, *G6P*; glucose 6-phosphate, *GAP*; glyceraldehyde 3-phosphate, *1,3 DPG*; 1,3-diphosphoglycerate, *NAD*$^+$; nicotinamide adenine dinucleotide (oxidized), *NADH*; nicotinamide adenine dinucleotide (reduced), *Oxa*; oxaloacetate, *Mal*; malate, *2-KGA*; 2-ketoglutarate, *Glu*; glutamate, *Asp*; aspartate, *CPT*; carnitine palmitoyltransferase, *CT*; carnitine acyltranslocase. CPT II is shown facing the matrix-side of the inner mitochondrial membrane

glycolytic pathway, the malate-aspartate shuttle for maintenance of high cytoplasmic $[NAD^+]/[NADH^+]$ ratios during high rates of glycolytic flux, a carnitine-dependent pathway for long-chain fatty acid oxidation, and high mitochondrial capacities for flux through the Krebs cycle, electron transport, proton pumping and oxidative phosphorylation (Suarez et al. 1986, 1991). It is both relevant and interesting to consider how a journal editor once commented that it was unfortunate that a new enzyme or pathway had not been found that explains why hummingbirds display such high metabolic rates. That evolution often works by making use of what is available through inheritance, rather than by reinventing biochemical machinery de novo whenever needed, should not be the cause of disappointment. In the case of the flight muscles of hummingbirds and bats, evolutionary processes have enhanced enzymatic flux capacities through known pathways of carbohydrate and fatty acid catabolism. Of particular interest is the upregulation of hexokinase (HK) activities; the resulting high capacities for glucose phosphorylation show the contrast between the flight muscles of hummingbirds and bat species with high sugar diets and those of birds of other species (Crabtree and Newsholme 1972b) and non-nectarivorous bats (Yacoe et al. 1982). However, hexokinase is not even considered to be a glycolytic enzyme by some biochemists (Fell 2000). It will be seen further on that the results of comparative physiology probably warrant a revision of this view.

Evolution has similarly enhanced pathways for long-chain fatty acid oxidation through increased enzyme levels. These are the same enzymes catalyzing reactions in the same pathway found in the red and cardiac muscles of other species of birds and mammals (Crabtree and Newsholme 1972a). Thus, carnitine palmitoyl transferase (CPT) activities (Fig. 1) in hummingbird flight muscles (Suarez et al. 1990) are extraordinarily high in comparison with other vertebrate skeletal muscles (Crabtree and Newsholme 1972a), and mitochondria isolated from this tissue display high rates of carnitine-dependent oxidation of palmitoylcoenzyme A (Suarez et al. 1986).

The biochemical evidence indicating high capacities for carbohydrate and fatty acid catabolism, mitochondrial respiration, and oxidative phosphorylation (Suarez et al. 1986, 1991) provide independent support of morphometric data (see below) that reveal extraordinarily high oxidative capacities in hummingbird flight muscles. But at what rates do these pathways actually operate in vivo?

## 3 Metabolic Rates During Flight

Hummingbirds and nectarivorous bats are capable of energetically expensive hovering, a mode of flight typically used to feed on floral nectar. Bartholomew and Lighton (1986) developed an elegant method for the measurement of $O_2$ consumption and $CO_2$ production rates during hover-feeding that has since been used in many studies of hummingbird flight energetics and adapted for use with nectarivorous bats. These respirometric data allow identification of the fuel(s) oxidized, as well as the estimation of flux rates through the relevant pathways of fuel oxidation (Suarez et al. 1990) and rates of ATP turnover (Welch et al. 2007). These

estimates also apply to forward flight because hummingbirds display relatively flat power curves (i.e., the energetic cost of flight is relatively constant) over a wide range of flight speeds (Berger 1985). Similarly, in small, nectarivorous bats, the power requirements for stationary hovering are close to those required for forward flight at intermediate speed (Voigt and Winter 1999).

Carbohydrate oxidation occurs with a respiratory quotient ($RQ = VCO_2/VO_2$) of 1.0, while fatty acid oxidation yields an RQ value of 0.71. In rufous hummingbirds, *Selasphorus rufus*, the species most extensively studied, hovering at $RQ = 1.0$ requires a rate of carbohydrate oxidation of 13.7 μmole glucosyl units per g muscle per minute (Suarez et al. 1990). On the other hand, the rate of fatty acid oxidation required to support hovering when $RQ = 0.71$ is 3.8 μmole palmitate per g muscle per minute.

These estimated flux rates can be compared with enzymatic flux capacities or $V_{max}$ values to yield further insights. However, further discussion should be preceded by a brief outline of some basic principles. $V_{max}$ values, measured in vitro under optimal conditions, equal $[E] \times k_{cat}$, where $[E]$ is enzyme concentration and $k_{cat}$ is the catalytic efficiency or turnover number of the enzyme molecules. The theory and rationale underlying comparisons between $V_{max}$ values and metabolic flux rates is well-developed and rigorous (Newsholme and Crabtree 1986) (but, unfortunately, commonly misunderstood). The measurement of $V_{max}$ values is not (and should not be) based on the naive and mistaken notion that metabolic enzymes necessarily operate at $V_{max}$ in vivo. Rather, $V_{max}$ values at non-equilibrium steps in pathways establish upper limits to flux, while the fractional velocities (i.e., fraction of $V_{max}$) at which the enzymes catalyzing these reactions operate are values that can be determined empirically. For example, the $V_{max}$ for glycogen phosphorylase in rufous hummingbird flight muscles is 31.2 μmole glucosyl units per g muscle per minute (Suarez et al. 1986). Although unremarkable in comparison with glycogen phosphorylase activities found in the muscles of other vertebrates (Crabtree and Newsholme 1972b), this is more than twofold higher than the required rate of carbohydrate oxidation (Suarez et al. 1990). However, HK $V_{max}$ values are extraordinarily high in rufous hummingbird flight muscles; if the oxidation of glucose (rather than glycogen) supports hovering flight, HK would operate at about 75% of its maximal capacity (Suarez et al. 1990). On the other hand, the $V_{max}$ for carnitine palmitoyl transferase is 7.2 μmole per g per minute (Suarez et al. 1990). It should be noted that this $V_{max}$ value was obtained using Triton X-100 during homogenization. Because there are two forms of CPT involved in fatty acid oxidation and this detergent inactivates one form (CPT I), but not the other (CPT II) (Woeltje et al. 1987), the $V_{max}$ value of 7.2 μmole per g per minute represents CPT II activity. Therefore, it is this form of the enzyme that operates at 47% of $V_{max}$. This exercise yields two important insights: The first is that high flux rates are made possible by high levels of enzyme expression at key steps in metabolism. The second is that, in addition to high flux capacities at these steps, some enzymes must operate at high fractional velocities (Suarez et al. 1997).

Although biochemical data specific to nectarivorous bats are not yet available, Yacoe et al. 1982 conducted a comparative biochemical study of enzymatic flux

capacities in the flight muscles of a number of bat species of varying diets and life histories. In addition to enzymatic data indicating generally high aerobic capacities, it was found that HK activities in fruit-eating (frugivorous) bats are in the neighborhood of $V_{max}$ values in rufous hummingbirds. In light of these data, it is reasonable to expect that HK activities in nectarivorous bats would be similarly high, and that mechanistic insights derived from hummingbirds might apply to these animals as well.

## 4 The Supply of Oxygen

During high-intensity, aerobic exercise, the steady-state rate of $O_2$ consumption of mitochondria in the locomotory musculature is equal to the rate of $O_2$ flux from the external environment through the respiratory and cardiovascular systems (Weibel 1984). Control of flux through the "oxygen transport cascade" is shared by various steps in the pathway of $O_2$ (di Prampero 2003; Jones 1998). Therefore, high rates of aerobic metabolism would not be expected to be based solely on the upregulation of biochemical capacities (Fig. 2). In hummingbirds, high rates of flux through the respiratory system are made possible, in part, by lung $O_2$-diffusing capacities higher than those of mammals of similar mass (Dubach 1981). Hummingbird hearts are much larger than what is predicted on the basis of the scaling of avian hearts (Bishop 1997). Their blood has high hematocrit, $O_2$-carrying capacity and -unloading efficiency (Johansen et al. 1987). Heart rates have been clocked at about 1,300 per minute (Lasiewski 1964), leading to estimates of cardiac outputs of five

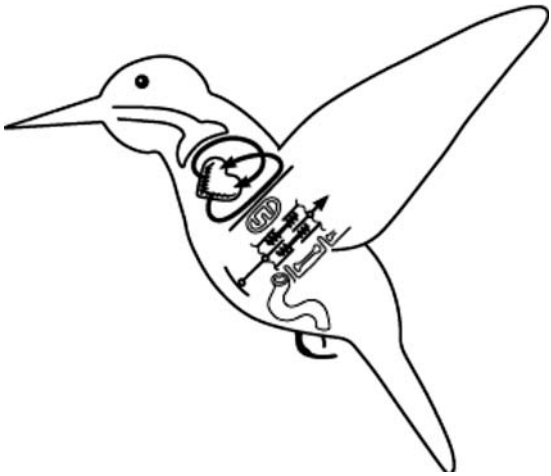

**Fig. 2** Diagram showing up-regulated parts of the hummingbird oxygen transport and sucrose oxidation cascades responsible for high rates of dietary energy intake and expenditure. It is redrawn from Suarez (1998) and was originally modified from a cartoon of a frog (Weibel 1985)

times body mass per minute and whole-body red blood cell circulation times of about 1 s (Johansen 1987). The flight muscles possess high capillary content that, in combination with small diameter muscle fibers, leads to high capacities for $O_2$ flux through the carrier-free zone between red blood cells and the muscle cell membranes (Mathieu-Costello et al. 1992; Suarez et al. 1991). Within the flight muscle fibers are found high myoglobin content (Johansen et al. 1987) and mitochondria occupying about 35% of cell volume (Suarez et al. 1991). These are among the highest observed among vertebrate locomotory muscles (Suarez 1996), while cristae surface areas of $58\,m^2$ per $cm^3$ of mitochondrial volume approach theoretical upper limits (Srere 1985). A significant fraction of the mitochondria are localized near the sarcolemma; the clustering of mitochondria close to capillaries may further increase capacities for $O_2$ flux (Mainwood and Rakusan 1982).

Bats are subject to the constraints imposed by mammalian evolutionary design. Nevertheless, studies concerning their cardiorespiratory systems reveal remarkable parallels with hummingbirds. These include larger hearts and cardiac outputs, high hematocrits and $O_2$-carrying capacities, pulmonary structures enhancing $O_2$ uptake, and high capillary densities in the flight muscles (reviewed in Maina 2000). Thus, hummingbirds and small bats are the natural analogues of human-engineered, high-performance automobiles possessing high-capacities for the delivery of both fuel and $O_2$.

## 5 Fuel Use During Flight: The Sucrose Oxidation Cascade

The high-energy density of triacylglycerol and its storage in unhydrated form (unlike glycogen) in muscles, liver and adipose tissue once led to the generalization that the energy for avian flight comes mainly from fatty acid oxidation (Blem 1976). In hummingbirds, long-term, migratory flight is preceded by premigratory fattening (Odum et al. 1961) and interrupted by refueling stops involving high rates of intake of dietary sucrose as well as high rates of fat synthesis (Carpenter and Hixon 1988; Carpenter et al. 1983). Migratory flight is known to deplete fat stores, so there is no doubt that most of the energy for hummingbird migration comes from fatty acid oxidation. Given high capacities for the oxidation of both carbohydrate and fat in hummingbird flight muscles (and, given the relatively minor contribution made by protein oxidation to vertebrate exercise metabolism), under what circumstances is each fuel used?

When they wake up after their overnight fast and hover to take their first sucrose meal in the morning, hummingbirds display RQ values close to 0.7, indicating that the flight muscles derive most of their energy from fatty acid oxidation (Suarez et al. 1990). Nectarivorous bats, on the other hand, fast in the daytime and feed at night. Recent results obtained with the nectar bat (*Glossophaga soricina*) also show RQ values close to 0.7 during the first feeding bouts after the daytime fast (Welch et al. 2008). In both hummingbirds and nectar bats, repeated hover-feeding visits to a sucrose dispenser (modified to function as a flow-through respirometer) result in

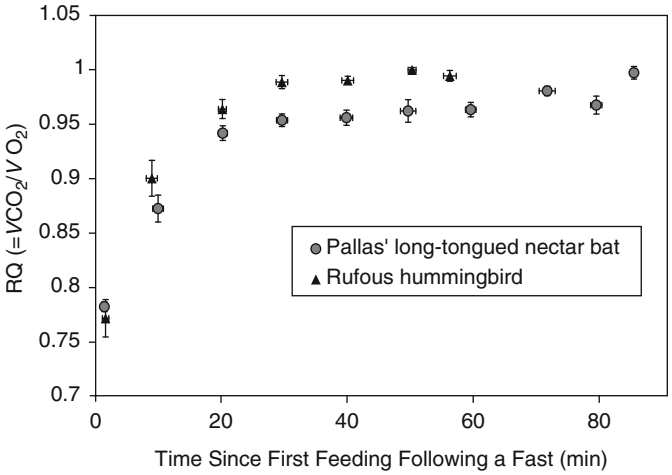


**Fig. 3** Respiratory quotient ($RQ = VCO_2/VO_2$) of rufous hummingbirds (*Selasphorus rufous*) and nectar bats (*Glossophaga soricina*) as a function of time after the first feeding bout following nighttime and daytime fasts respectively. Fasting results in RQ values close to 0.7, indicating fatty acid oxidation. Repeated hover-feeding results in rapid increases to values close to 1.0, indicating switching from fatty acid oxidation to carbohydrate oxidation. Points represent means and standard errors. Data are from Welch et al. (2008)

rapid, progressive increases in RQ values until these stabilize to about 1.0, indicating that carbohydrate serves as the main oxidative fuel for flight when dietary sucrose is available (Suarez et al. 1990; Welch et al. 2008, b) (Fig. 3). But what is the nature of this carbohydrate?

To address this question, experiments were conducted that took advantage of the difference in $^{13}C/^{12}C$ ratios of sucrose molecules produced by plants through C3 (sugar beet) or C4 (sugar cane) photosynthesis. Beet sugar contains a lower $^{13}C/^{12}C$ ratio (expressed relative to a standard as $\delta^{13}C$) than cane sugar. Thus, hummingbirds and nectar bats reared on a diet containing beet sugar incorporate beet sugar carbon into their stores of carbohydrate and fat, and soon expire $CO_2$ with a low $\delta^{13}C$, reflecting that of beets. When these animals are fasted and then allowed to hover-feed, their RQ values are initially close to 0.7, as expected, while the $\delta^{13}C$ value of their expired $CO_2$ remains low and "beet-like". In these experiments, the fasted animals are then provided cane sugar, which has a higher $\delta^{13}C$ resulting from C4 photosynthesis. As the RQ rises during repeated feeding from about 0.7 to 1.0, the $\delta^{13}C$ also increases from values reflecting the oxidation of fat made from beet sugar to higher $\delta^{13}C$ values indicating the direct use of recently ingested cane sugar (Welch et al. 2006, 2008) (Fig. 4). Calculations based on these results reveal that close to 100% of the fuel oxidized during repeated foraging in hummingbirds comes directly from recently ingested sucrose, while recently ingested sucrose fuels close to 80% of hovering metabolism in the case of nectar bats.

The high flux rates from dietary sucrose (a disaccharide consisting of glucose and fructose) to expired $CO_2$ inspire comparison with the oxygen transport cascade,

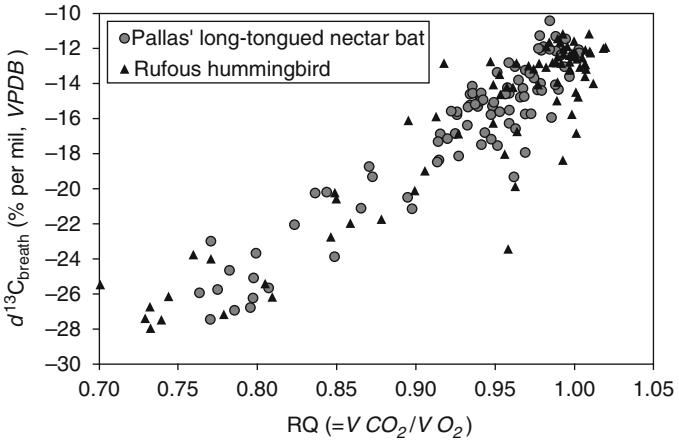


**Fig. 4** $\delta^{13}C$ values of expired $CO_2$ in hovering breath samples collected from rufous hummingbirds (*Selasphorus rufous*) and nectar bats (*Glossophaga soricina*), plotted as a function of RQ. Low $\delta^{13}C$ values when RQ $\approx$ 0.7 result from the lower $^{13}C/^{12}C$ ratio of beet sugar provided in the maintenance diet, while high $\delta^{13}C$ values when RQ $\approx$ 1.0 result from the $^{13}C/^{12}C$ ratio of cane sugar provided in experiments. The increase in $\delta^{13}C$ values as RQ goes from 0.7 to 1.0 indicates that recently ingested dietary sugar accounts for most of the carbohydrate oxidized during hover-feeding. Points represent individual measurements. Data are from Welch et al. (2008)

i.e., the pathway of $O_2$ from the external environment, through the respiratory and cardiovascular systems, to the muscle mitochondria where reduction of the oxygen atoms to $H_2O$ takes place. In the oxygen transport cascade, the driving force for $O_2$ flux is the large $pO_2$ gradient between the external environment and the mitochondria, which serve as $O_2$ sinks. In the case of dietary sugar, the "sucrose oxidation cascade" involves high rates of ingestion, hydrolysis by intestinal sucrase (McWhorter and Martinez del Rio 2000), transport across the intestinal epithelium via both carrier-mediated and paracellular pathways (McWhorter et al. 2006), entry into the blood and, ultimately, oxidation in the mitochondria which serve as carbon sinks that convert organic carbon molecules derived from dietary sucrose into $CO_2$. The pathway is not as simple as in the case of $O_2$. In humans, about half of ingested fructose is converted to glucose by the liver which then appears in the blood (Delarue et al. 1993). Skeletal muscles possess lower capacities for fructose transport (Kristiansen et al. 1997) and oxidation (Zierath et al. 1995) relative to glucose. Both glucose and fructose can serve as substrates for HK-catalyzed phosphorylation, yielding glucose 6-phosphate and fructose 6-phosphate, respectively. Thus, irrespective of whether hummingbird and nectar bat flight muscles directly oxidize dietary fructose at significant rates or not, the stable carbon isotope experiments provide further evidence that most carbon must go through the HK step in hummingbirds and nectar bats as they fuel foraging flights with carbohydrate. An unresolved issue concerns the carbon fluxes to and from glycogen. Given the high metabolic rates fueled by carbohydrate, it is likely that liver and muscle glycogen pools would not last long (about 5 min, at most) if these animals were to rely exclusively

on glycogen to fuel flight (Suarez et al. 1990). In addition, the carbon stable isotope results reveal that the exogenous sugar turns over rapidly within the pool of metabolized substrates (Welch and Suarez 2007). Thus, a viable working hypothesis is that liver glycogen serves to buffer blood glucose concentrations while flight muscle glycogen buffers the pool of hexose-phosphates in glycolysis during foraging flights. In this scenario, muscle glycogen phosphorylase and glycogen synthase fluxes would change dynamically and in reciprocal fashion, but all sucrose-derived carbon appearing in the blood would still have to go through the HK reaction in exercising muscles. These animals, while ingesting sucrose and expiring sucrose-derived $CO_2$ at high rates, now begin to appear more as the natural analogues of high-performance aircraft engaged in aerial refueling. It is interesting to consider how, in nature, this aerial refueling contributes to plant pollination and plant biodiversity.

## 6 In Vitro, In Vivo and Beyond

According to Chantler (1982), "the most noble aim of the biochemist, often discussed when inebriate, seldom when sober, is to relate the in vitro to the in vivo". Studies concerning hummingbirds and, more recently, nectar bats have revealed that the highest rates of aerobic metabolism observed among vertebrates result from the concerted upregulation of capacities for fuel and $O_2$ fluxes in multiple organ systems and at multiple levels of biological organization. At the biochemical level, key enzymes are expressed at higher concentrations and operate at higher fractional velocities than in other species. But Chantler's dictum can be taken even further, and into the realm of ecology and evolution. Premigratory rufous hummingbirds are known for their ability to ingest sucrose at rates high enough to enable fat deposition at rates of up to 10% of body mass per day (Carpenter and Hixon 1988; Carpenter et al. 1983). Their livers possess high acetylcoenzyme A carboxylase activities (Suarez et al. 1988), allowing dietary sucrose in excess of daily energy needs to be rapidly converted to fatty acids and esterified to fat. However, fat synthesis costs ATP, so it appears that one way hummingbirds behave "efficiently" to maximize net energy gain is to engage in short foraging bouts and to oxidize dietary sugar. This avoids the use of fat, but also avoids the energetic inefficiency of synthesizing fat from dietary sucrose, and then using fat to fuel foraging flight. This implies that the greater energetic efficiency resulting from the direct use of dietary sugar, and the consequent enhancement of net daily energy gain, might be one of the benefits of territorial behavior. If this is the case, an intriguing suggestion is that metabolic biochemistry may have contributed to the evolution of optimal foraging behavior or even the evolution of territoriality in hummingbirds (Suarez and Gass 2002).

The latest estimates of the number of ATP molecules synthesized per oxygen atom consumed (i.e., the *P/O* ratio) indicate that about 15% less oxygen is required per mole of ATP synthesized when glucose is oxidized as compared with fatty acid oxidation (Brand 2005). Part of the mechanistic basis for this difference can be seen

in the difference in *P/O* ratios displayed by isolated, coupled mitochondria isolated from hummingbird flight muscles oxidizing pyruvate, as compared with palmitoyl-CoA plus carnitine (Suarez et al. 1986). An interesting question is whether this substrate-dependent difference in *P/O* ratio makes any difference to whole animals in the context of what they do in nature. A given mass of hummingbird requires the same amount of energy to hover, irrespective of whether its flight muscles oxidize carbohydrate or fat. This leads to the prediction that hovering $VO_2$ should decline by 15% as hummingbirds transition from the fasted $(RQ = 0.71)$ to the fed $(RQ = 1.0)$ state. Recent data obtained using rufous and Annas (*Calypte anna*) hummingbirds support this hypothesis (Welch et al. 2007); this is the first report of the direct influence of substrate-dependent *P/O* ratios on whole animal performance. Migratory rufous hummingbirds refuel at high-altitude, subalpine meadows where they experience the combined effects of low air density and hypoxia (Gass et al. 1999; Welch and Suarez 2008). Thus, territorial hummingbirds maximizing net energy intake by oxidizing dietary sugar may derive additional benefits at high altitude from the 15% lower requirement for $O_2$.

## 7 Concluding Remarks

It seems unlikely that there would be reductionist, single gene, or single enzyme-based explanations for how nature's fires of life burn so brightly in hummingbirds and nectar bats. Rather, these amazing animals are the result of the concerted upregulation of flux capacities in multiple organ systems and multiple levels of organization. This suggests that a quantitative, integrative "systems approach" would be more fruitful in advancing understanding than simple reductionism. The well-known, highly conserved and modular nature of metabolic pathways among vertebrates may suggest that the study of vertebrate metabolic biochemistry is no longer interesting or worthwhile, except when applied to human disease states. On the contrary, the great reliance on dietary sucrose as a direct fuel for exercise metabolism during hummingbird and nectar bat foraging is an excellent illustration of the significance of quantitative variation in biochemical flux capacities across species. Studies of intermediary metabolism and its regulation in these animals may yield useful insights into diseases that tend to afflict humans who eat too much carbohydrate, do not exercise and deposit too much fat.

Comparative physiology involves the exploration of functional biodiversity in the natural world. At the core of this research program are studies of functional integration in ecological and evolutionary contexts. The work on hummingbirds has led to insights concerning the ultimate (evolutionary) upper limits to aerobic capacities (Suarez 1998) as well as information concerning the environmental factors and responses to them that allow (or prevent) energy balance or net energy gain (Gass et al. 1999; Suarez and Gass 2002). It is not difficult to imagine how the outcome of studies at the interfaces between physiology, biochemistry and ecology

might prove useful, especially in a period characterized by declining biodiversity and global climate change.

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## References

Bartholomew GA, Lighton JRB (1986) Oxygen consumption during hover-feeding in free-ranging Anna hummingbirds. Journal of Experimental Biology 123:191–199

Berger M (1985) Sauerstoffverbrauch von Kolibris (*Colibri coruscans* und *C. thalassinus*) beim Horizontalflug. BIONA Report 3. In: W Nachtigall (ed) Biona-Report 3, Bird Flight. G Fischer, Stuttgart, pp 307–314

Bishop CM (1997) Heart mass and the maximum cardiac output of birds and mammals: implications for estimating the maximum aerobic power input of flying animals. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 352:447–456

Blem CR (1976) Patterns of lipid storage and utilization in birds. American Zoologist 16:671–684

Blomstrand E, Ekblom B, Newsholme EA (1986) Maximum activities of key glycolytic and oxidative enzymes in human muscle from differently trained individuals. Journal of Physiology 381:111–118

Brand MD (2005) The efficiency and plasticity of mitochondrial energy transduction. Biochemical Society Transactions 33:897–904

Carpenter FL, Hixon MA (1988) A new function for torpor: fat conservation in a wild migrant hummingbird. Condor 90:373–378

Carpenter FL, Paton DC, Hixon MA (1983) Weight gain and adjustment of feeding territory size in migrant hummingbirds. Proceedings of the National Academy of Sciences of the United States of America 80:7259–7263

Chai P, Dudley R (1995) Limits to vertebrate locomotor energetics suggested by hummingbirds hovering in heliox. Nature 377:722–725

Chantler PD (1982) Caged ATP set free in muscle. Nature 300:682–683

Crabtree B, Newsholme EA (1972a) The activities of lipases and carnitine palmitoyltransferase in muscles from vertebrates and invertebrates. The Biochemical Journal 130:697–705

Crabtree B, Newsholme EA (1972b) The activities of phosphorylase, hexokinase, phosphofructokinase, lactate dehydrogenase and the glycerol 3-phosphate dehydrogenases in muscles from vertebrates and invertebrates. The Biochemical Journal 126:49–58

Delarue J, Normand S, Pachiaudi C, Beylot M, Lamisse F, Riou JP (1993) The contribution of naturally labelled $^{13}C$ fructose to glucose appearance in humans. Diabetologia 36:338–345

di Prampero PE (2003) Factors limiting maximal performance in humans. European Journal of Applied Physiology 90:420–429

Dubach M (1981) Quantitative analysis of the respiratory system of the house sparrow, budgerigar and violet-eared hummingbird. Respiration Physiology 46:43–60

Dudley R (2000) The Biomechanics of Insect Flight. Princeton University Press, Princeton

Epting RJ (1980) Functional dependence of the power for hovering on wing disc loading in hummingbirds. Physiological Zoology 53:347–357

Fell DA (2000) Signal transduction and the control of expression of enzyme activity. Advances in Enzyme Regulation 40:35–46

Fons R, Sicart R (1976) Contribution à la connaissance du metabolisme énergétique chez deux crocidurinae: *Suncus etruscus* (Savi, 1822) et *Crocidura russula* (Hermann, 1780) (Insectivora, Soricidae). Mammalia 40:299–311

Gass CL, Romich MT, Suarez RK (1999) Energetics of hummingbird foraging at low ambient temperature. Canadian Journal of Zoology 77:314–320
Grinyer I, George JC (1969) Some observations on the ultrastructure of the hummingbird pectoral muscles. Canadian Journal of Zoology 47:771–774
Johansen K (1987) The world as a laboratory: physiological insights from Nature's experiments. In: McLennan H, Ledsome JR, McIntosh CHS (eds) Advances in Physiological Research. Plenum Press, New York, pp 377–396
Johansen K, Berger M, Bicudo JEPW, Ruschi A, De Almeida PJ (1987) Respiratory properties of blood and myoglobin in hummingbirds. Physiological Zoology 60:269–278
Jones JH (1998) Optimization of the mammalian respiratory system: symmorphosis versus single species adaptation. Comparative Biochemistry and Physiology 120B:125–138
Kleiber M (1961) The Fire of Life. Wiley, New York
Kristiansen S, Darakhshan F, Richter EA, Handal HS (1997) Fructose transport and GLUT5 protein in human sarcolemmal vesicles. American Journal of Physiology 273:E543–E548
Lasiewski RC (1963) Oxygen consumption of torpid, resting, active, and flying hummingbirds. Physiological Zoology 36:122–140
Lasiewski RC (1964) Body temperatures, heart and breathing rate, and evaporative water loss in hummingbirds. Physiological Zoology 37:212–223
Lasiewski RC, Galey FR, Vasquez C (1965) Morphology and physiology of the pectoral muscles of hummingbirds. Nature 205:404–405
Maina JN (2000) What it takes to fly: the structural and functional respiratory requirements in birds and bats. Journal of Experimental Biology 203:3045–3064
Mainwood GW, Rakusan K (1982) A model for intracellular energy transport. Canadian Journal of Physiology and Pharmacology 60:98–102
Mathieu-Costello O, Suarez RK, Hochachka PW (1992) Capillary-to-fiber geometry and mitochondrial density in hummingbird flight muscle. Respiratory Physiology 89:113–132
McWhorter TJ, Martinez del Rio C (2000) Does gut function limit hummingbird food intake? Physiological and Biochemical Zoology 73:313–324
McWhorter TJ, Bakken BH, Karasov WH, Martinez del Rio C (2006) Hummingbirds rely on both paracellular and carrier-mediated intestinal glucose absorption to fuel high metabolism. Biology Letters 2:131–134
Newsholme EA, Crabtree B (1986) Maximum catalytic activity of some key enzymes in provision of physiologically useful information about metabolic fluxes. Journal of Experimental Zoology 239:159–167
Odum EP, Connell CE, Stoddard HL (1961) Flight energy and estimated flight ranges of some migratory birds. Auk 78:515–527
Rosser BWC, George JC (1986) The avian pectoralis: histochemical characterization and distribution of muscle fiber types. Canadian Journal of Zoology 64:1174–1185
Srere PA (1985) Organization of proteins within the mitochondrion. In: Welch GR (ed) Organized Multienzyme Systems Catalytic Properties. Academic Press, New York, pp 1–61
Suarez RK (1992) Hummingbird flight: sustaining the highest mass-specific metabolic rates among vertebrates. Experientia 48:565–570
Suarez RK (1996) Upper limits to mass-specific metabolic rates. Annual Review of Physiology 58:583–605
Suarez RK (1998) Oxygen and the upper limits to animal design and performance. Journal of Experimental Biology 201:1065–1072
Suarez RK, Gass CL (2002) Hummingbird foraging and the relation between bioenergetics and behaviour. Comparative Biochemistry and Physiology Part A 133:335–343
Suarez RK, Brown GS, Hochachka PW (1986) Metabolic sources of energy for hummingbird flight. American Journal of Physiology 251:R537–R542
Suarez RK, Brownsey RW, Vogl W, Brown GS, Hochachka PW (1988) Biosynthetic capacity of hummingbird liver. American Journal of Physiology 255:R699–R702

Suarez RK, Lighton JRB, Moyes CD, Brown GS, Gass CL, Hochachka PW (1990) Fuel selection in rufous hummingbirds: ecological implications of metabolic biochemistry. Proceedings of the National Academy of Science U S A 87:9207–9210

Suarez RK, Lighton JRB, Brown GS, Mathieu-Costello O (1991) Mitochondrial respiration in hummingbird flight muscles. Proceedings of the National Academy of Science U S A 88: 4870–4873

Suarez RK, Lighton JRB, Joos B, Roberts SP, Harrison JF (1996) Energy metabolism, enzymatic flux capacities and metabolic flux rates in flying honeybees. Proceedings of the National Academy of Science U S A 93:12616–12620

Suarez RK, Staples JF, Lighton JRB, West TG (1997) Relationships between enzymatic flux capacities and metabolic flux rates in muscles: nonequilibrium reactions in muscle glycolysis. Proceedings of the National Academy of Science U S A 94:7065–7069

Taylor CR (1987) Structural and functional limits to oxidative metabolism: Insights from scaling. Annual Review of Physiology 49:135–146

Voigt CC, Winter Y (1999) Energetic cost of hovering flight in nectar-feeding bats (Phyllostomidae: Glossophaginae) and its scaling in moths, birds and bats. Journal of Comparative Physiology 169:38–48

Weibel ER (1984) The Pathway for Oxygen. Harvard University Press, Cambridge, MA, USA

Weibel ER (1985) Design and performance of muscular systems: an overview. Journal of Experimental Biology 115:405–412

Welch Jr. KC, Bakken BH, Martinez del Rio C, Suarez RK (2006) Hummingbirds fuel hovering flight with newly-ingested sugar. Physiological and Biochemical Zoology 79:1082–1087

Welch Jr. KC, Suarez RK (2007) Oxidation rate and turnover of ingested sugar in hovering Anna's (*Calypte anna*) and rufous (*Selasphorus rufus*) hummingbirds. Journal of Experimental Biology 210:2154–2162

Welch Jr. KC, Suarez RK (2008) Altitude and temperature effects on the energetic cost of hoverfeeding in migratory rufous hummingbirds, *Selasphorus rufus*. Canadian Journal of Zoology 86:161–169

Welch Jr. KC, Altshuler DL, Suarez RK (2007) Oxygen consumption rates in hovering hummingbirds reflect substrate-dependent differences in *P/O* ratios: carbohydrate as a 'premium fuel'. Journal of Experimental Biology 210:2146–2153

Welch Jr. KC, Herrera LG, Suarez RK (2008) Dietary sugar as a direct fuel for flight in the nectarivorous bat, *Glossophaga soricina*. Journal of Experimental Biology 211:310–316

Winter Y, Voigt C, von Helversen O (1998) Gas exchange during hovering flight in a nectar-feeding bat *Glossophaga soricina*. Journal of Experimental Biology 201:237–244

Woeltje KF, Kuwajima M, Foster DW, McGarry JD (1987) Characterization of the mitochondrial carnitine palmitoyltransferase enzyme system. I. Use of detergents and antibodies. Journal of Biological Chemistry 262:9822–9827

Yacoe ME, Cummings JW, Myers P, Creighton GK (1982) Muscle enzyme profile, diet and flight in South American bats. American Journal of Physiology 242:R189–R194

Zierath JR, Nolte LA, Wahlstrom E, Galuska D, Shepherd PR, Kahn BB, Wallberg-Henriksson H (1995) Carrier-mediated fructose uptake significantly contributes to carbohydrate metabolism in human skeletal muscle. Biochemistry Journal 311:517–521