Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9207–9210, December 1990
Ecology

Fuel selection in rufous hummingbirds: Ecological implications of metabolic biochemistry

(energy metabolism/metabolic rate/carbohydrate oxidation/fatty acid oxidation/foraging strategy)

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Communicated by Jared M. Diamond, August 20, 1990

ABSTRACT **Hummingbirds in flight display the highest rates of aerobic metabolism known among vertebrates. Their flight muscles possess sufficient maximal activities of hexokinase and carnitine palmitoyltransferase to allow the exclusive use of either glucose or long-chain fatty acids as metabolic fuels during flight. Respiratory quotients (RQ = V_{CO_2}/V_{O_2}) indicate that fatty acid oxidation serves as the primary energy source in fasted resting birds, while subsequent foraging occurs with a rapid shift towards the use of carbohydrate as the metabolic fuel. We suggest that hummingbirds building up fat deposits in preparation for migration behave as carbohydrate maximizers (or fat minimizers) with respect to the metabolic fuels selected to power foraging flight.**

Hummingbirds display extremely high mass-specific metabolic rates (rates of O_2 consumption per unit body mass, V_{O_2}/M_b) at rest and in flight (1–4). A question that constantly arises in view of their phenomenal rate of aerobic metabolism and nectarivorous diet is the nature of the metabolic fuels oxidized under various conditions—e.g., fasting, short-term foraging, and long-term migratory flight. A further question concerns the factors determining which fuel is used when. We have addressed these issues through the combined use of respirometry and the measurement of maximal activities of regulatory (and potentially rate-determining) enzymes in energy metabolism. The former approach allows assessment of metabolic rates as well as the nature of the fuel(s) used to achieve these rates (5); the latter provides estimates *in vitro* of maximum possible rates of metabolic flux that can be compared with actual flux rates *in vivo* calculated from V_{O_2}/M_b (6).

MATERIALS AND METHODS

Rufous hummingbirds (*Selasphorus rufus*) were captured and maintained and rates of O_2 consumption were measured as previously described (1, 7). The method was slightly modified to allow measurement of CO_2 production by using an Anarad AR 400 infrared gas analyzer. Data acquisition and analysis were done with Datacan IV (Sable Systems, Los Angeles).

Samples were prepared for enzyme measurements as previously described (7) with the following modifications: Triton X-100 (1% vol/vol) was included in the homogenization medium and homogenates were sonicated (10 s three times) before centrifugation. Enzyme assays were conducted as described previously (7).

RESULTS AND DISCUSSION

Rates of gas exchange were measured in quiescent birds fasted for 1–2 h and held in cloth jackets in the dark, birds feeding while perched, and birds hovering to feed (Fig. 1). Almost all hover-feeding bouts lasted less than 10 s, although bouts as long as 33 s were observed. Resting V_{O_2}/M_b was about 10 ml/(g·h), and it increased 2.5-fold during feeding while perched, and 3.8-fold during hovering (Table 1). Our "resting" metabolic rates are about 2-fold higher than those reported by Lasiewski (4) and are in close agreement with measurements made more recently by Bucher and Chappell (3), who used unrestrained animals. V_{O_2}/M_b values during hover-feeding are similar to those reported previously (1). These data imply about a 4-fold activation of aerobic metabolism during the rest-to-flight transition, rather than 10-fold as reported previously (4). The hovering and perching metabolic rates reported here (Table 1) are easily supported by the energy intake rates known in wild hummingbirds. During postbreeding migration, territorial rufous hummingbirds average 1.6 kJ/h (0.45 W) gross nectar intake over a 14-h foraging day (8, 9). This approximates existence metabolism (10) and is enough to fuel foraging, territory defense, and thermoregulation, as well as to support rates of fat deposition observed in the wild (11).

RQ values obtained with fasted, resting birds were about 0.72, indicating the use of fat as the major metabolic fuel (5). Birds in the process of licking sugar water while perched

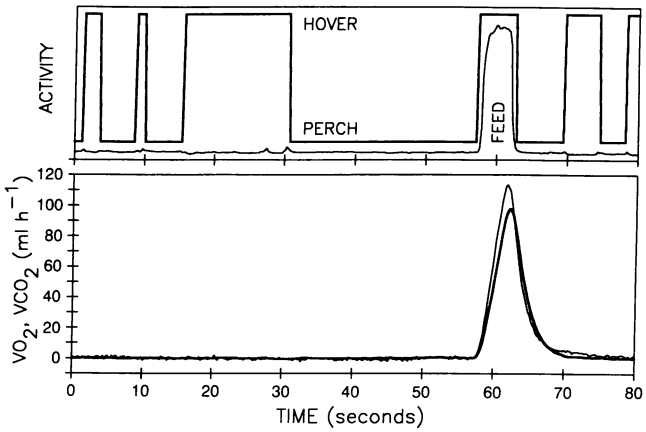

FIG. 1. Typical data obtained from a rufous hummingbird, mass 3.29 g, hovering while feeding from a sugar-water dispenser modified to function as a flow-through respiratory mask (1). (*Lower*) Rates of O_2 production (V_{O_2}; thin trace) and CO_2 production (V_{CO_2}; thick trace). Integration beneath the peaks yields volumes of O_2 and CO_2 exchanged by the hovering bird while its nares were fully within the mask, determined photoelectrically (4.39 s; thin trace in *Upper*). Hovering activity was also monitored by using a perch switch (thick trace in *Upper*). Dividing O_2 and CO_2 volumes by duration of mask occupancy yields V_{O_2} and V_{CO_2} [37.0 and 33.1 ml/(g·h), respectively, when divided by the mass of the bird]. H_2O was scrubbed from the airstream prior to analysis of CO_2 content; both H_2O and CO_2 were scrubbed from the airstream prior to measurement of O_2 content and flow rate. Not all birds hovered with their nares deep enough within the mask to yield good data on metabolic rate while hovering, although all birds yielded good respiratory quotient (RQ) data.

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Table 1. Metabolic rates and respiratory quotients (RQ values) in restrained, perch-feeding, and hovering hummingbirds

State	Vo_2/M_b, ml/(g·h)	RQ	W/kg	W per bird
Restrained*	10.1 ± 0.4	0.72 ± 0.01†	55.3 ± 2.4	0.23 ± 0.01
Perch-feeding‡	27.2 ± 1.3	0.96 ± 0.03	158.0 ± 7.4	0.52 ± 0.02
Hover-feeding	38.3 ± 1.1§	1.02 ± 0.01¶	225.9 ± 6.7	0.76 ± 0.02

Values were obtained at a room temperature of 25°C and are means ± SEM. Vo_2/M_b is expressed as ml of O_2/(g of bird·h). RQ = Vco_2/Vo_2. W/kg (W = watts) is calculated from [(RQ × 5.164) + 15.97] × [Vo_2/M_b] × [(1000 g/kg)/(3600 s/h)] (18). W per bird is calculated from W/kg and mean mass.
*21 measurements on 15 birds with mean mass of 3.89 g.
†Significantly different from RQ values obtained during perch-feeding and hover-feeding ($P < 0.0001$).
‡18 measurements on 6 birds with mean mass of 3.28 g.
§10 measurements on 4 birds with mean mass of 3.38 g.
¶72 measurements on 9 birds with mean mass of 3.44 g.

oxidized carbohydrate (RQ = 0.96). The first bout of hover-feeding after a 1- to 2-h fast occurred with an RQ of about 0.81, while subsequent bouts gave RQ values of about 1.0 (Fig. 2), indicating a shift towards carbohydrate oxidation during repeated foraging after fasting.

The hummingbird's ability to use either carbohydrate or fat to power flight is made possible by extremely high enzymatic capacities for flux through pathways of carbohydrate and fatty acid oxidation (7). In the present study, we have employed an improved extraction procedure that yields higher maximal activities of hexokinase and carnitine palmitoyltransferase (regulatory enzymes involved in glucose and long-chain fatty acid oxidation, respectively) (Table 2) than values reported previously (7). Citrate synthase (a Krebs cycle enzyme) was also measured; maximal activities of this enzyme were about 30% higher [448.4 ± 27.8 μmol/(g·min), mean ± SEM, $n = 6$] than previously reported (7). These maximal enzyme activities are, to our knowledge, the highest reported for vertebrate skeletal muscles and can readily support the rates of glucose or fatty acid oxidation [equivalent to ATP turnover rates of 492 μmol per g of flight muscle per min (see legend of Table 2)] required for hovering and forward flight. The estimated flux rates through the hexokinase and carnitine palmitoyltransferase reactions during flight are close to the maximum catalytic capacities measured (Table 2), indicating that despite the great degree of up-regulation of these enzymes, not much "excess capacity" is present at these steps in hummingbird flight muscle energy metabolism. From these data and those previously reported (7), a map of the "organization" of energy metabolism in hummingbird flight muscles is presented (Fig. 3). According to this model, carbohydrate-based aerobic metabolism is fueled by endogenous glycogen in muscles and glucose from the blood, crop, liver, and gut. Cytosolic redox balance ($NADH/NAD^+$ ratio) is probably maintained via the malate–aspartate shuttle, since extremely high activities of both aspartate transaminase and malate dehydrogenase but relatively low activities of α-glycerophosphate dehydrogenase are found (7). Fatty acids enter hummingbird flight muscle mitochondria through the same carnitine-dependent process described in other vertebrate animals, and they supply acetyl-coenzyme A units to the Krebs cycle via β-oxidation (7).

It has been suggested previously that hummingbirds may be energy "maximizers" that "take in energy as rapidly as their digestive processes permit" (12). During premigratory fattening, lipid deposition rates of between 0.3 and 0.5 g per day have been observed in birds weighing between 3 and 5 g (11, 13). This is achieved by a combination of behavioral and biochemical adaptations that may include the conservation of energy via torpor (13), the optimization of territory size (11), the highest rates of intestinal glucose absorption known (12, 14), and a hepatic fatty acid biosynthetic capacity at least 10-fold greater than that found in mammals (15).

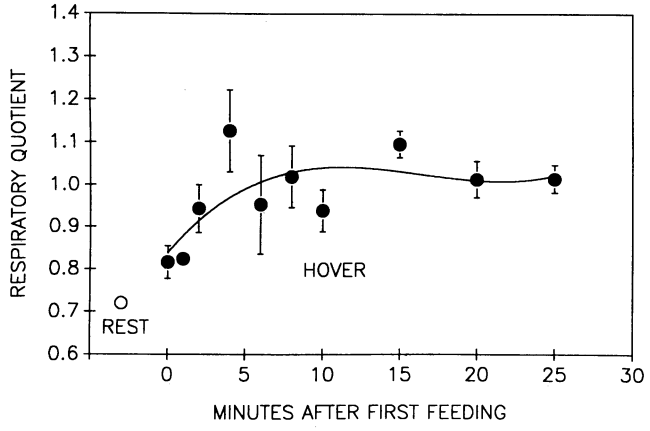

FIG. 2. RQ (Vco_2/Vo_2) during hovering flight after 1–2 h of fasting. RQ at first feeding bout = 0.81 ± 0.04 ($n = 9$ measurements on 9 birds). Following first feeding, RQ increased to 1.02 ± 0.01 ($n = 72$ measurements on 9 birds). The increase was significant ($t = 5.1$; $P < 0.0001$). Value determined from resting (restrained, fasted) birds is denoted by the open circle. All points denote means; error bars denote SEM.

Table 2. Comparison of required substrate oxidation rates in flight muscles of flying hummingbirds with maximum possible rates of flux through hexokinase and carnitine palmitoyltransferase

Substrate	Oxidation rate, μmol/(g·min)	
	Required	Maximum possible
Glucose	13.7*	18.4 ± 1.38†
Palmitate	3.8‡	3.6 ± 0.19§
Oleate	3.4¶	3.6 ± 0.19§

Values are in μmol of substrate required for oxidation to CO_2 and H_2O per g of muscle per min to account for an ATP turnover rate of 492 μmol/(g·min) (first column) or maximal enzyme activities (μmol of substrate converted to product per g of muscle per min) (second column). ATP turnover rate is calculated as follows: One gram of flight muscle in a hummingbird consuming 38 ml of O_2 per g of bird per h during flight would consume about 2 ml of O_2/min or 82 μmol of O_2/min. This is equivalent to an ATP turnover rate of 492 μmol/min, assuming a P/O_2 ratio of 6.
*Based on production of 36 μmol of ATP as a result of oxidation of 1 μmol of glucose.
†Based on maximal activities of hexokinase (mean ± SEM, $n = 5$) at 39°C.
‡Based on production of 129 μmol of ATP as a result of oxidation of 1 μmol of palmitate.
§Based on maximal activities of carnitine palmitoyltransferase at 39°C divided by 2, since there are 2 compartmentalized forms of the enzyme (mean ± SEM, $n = 6$).
¶Based on production of 144 μmol of ATP as a result of oxidation of 1 μmol of oleate.

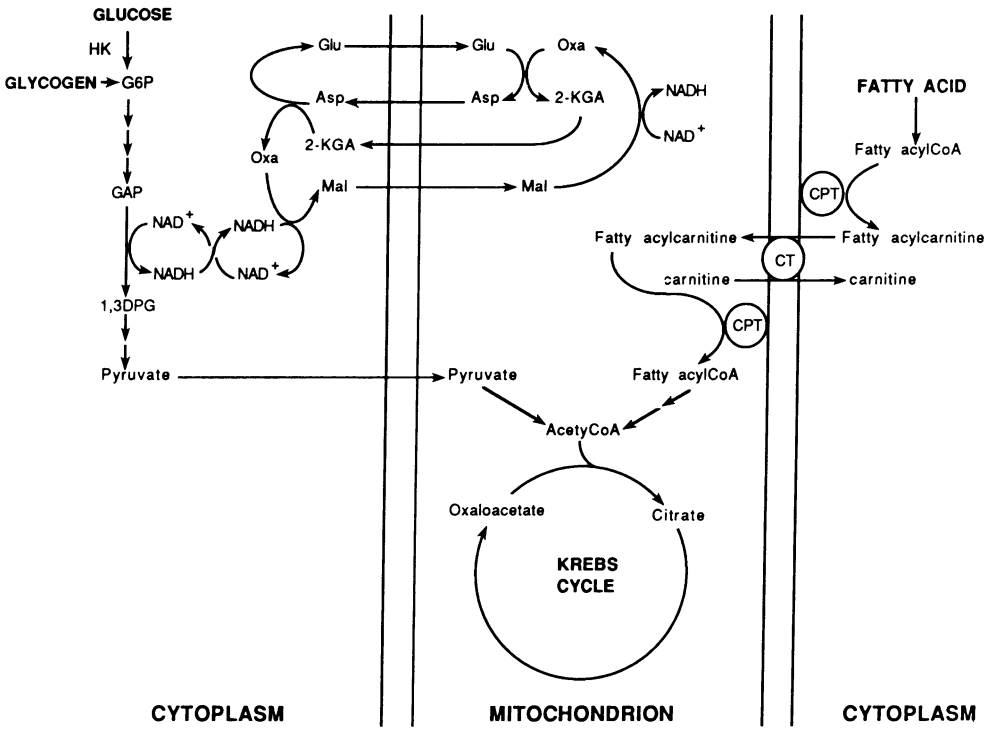

FIG. 3. Pathways of carbohydrate (left) and fatty acid (right) oxidation in hummingbird flight muscles. Carbohydrate oxidation predominates as the major energy source during short-term foraging flight. Cytosolic redox balance ($NADH/NAD^+$) is maintained during carbohydrate oxidation by transfer of reducing equivalents into mitochondria by the malate–aspartate shuttle. Lipid stores are mobilized during long-term and migratory flight and fatty acid oxidation results in inhibition of carbohydrate oxidation. HK, hexokinase; CPT, carnitine palmitoyltransferase; CT, carnitine acyltranslocase; G6P, glucose 6-phosphate; GAP, glyceraldehyde 3-phosphate; 1,3DPG, 1,3-diphosphoglycerate; 2-KGA, 2-ketoglutarate; Mal, malate; Oxa, oxaloacetate.

We suggest, in addition, that the optimal metabolic strategy for hummingbirds accumulating fat in preparation for migratory flight would be to behave as "carbohydrate maximizers" (or "fat minimizers"), i.e., to preferentially oxidize carbohydrate (not fat) during foraging. A 3- to 4-g hummingbird has about 0.1 g of liver (15) and 1 g of flight muscles (7), containing about 40 and 30 μmol of glucosyl units stored as glycogen, respectively (assuming "typical" vertebrate glycogen contents). Total depletion of these glycogen stores, though highly unlikely, would provide carbon for aerobic metabolism for a maximum of about 5 min at the metabolic rates estimated during flight (Table 2). By keeping foraging bouts well under 5 min, hummingbirds are able to rely upon carbohydrate as their major oxidative fuel and spare their fat stores. Indeed, Gass and Sutherland (9) have reported that foraging bout duration in the field is about 10% of this theoretical carbohydrate-fueled maximum. Such a strategy increases both the *rate* at which net fat deposition is achieved and the *efficiency* with which dietary carbon is utilized. This efficiency advantage results from the energetic cost of fatty acid synthesis from dietary glucose. Synthesis of fatty acids from glucose before oxidation results in a 16% lower *net* yield of ATP compared with direct oxidation of dietary glucose without further processing.¶ Thus, although there is sufficient capacity to oxidize fatty acid to account for hovering and forward flight (Table 2), and it is known that fat is the major fuel used during long-term migratory flight (17), it is more advantageous for hummingbirds to rely upon carbohydrate to provide the energy for foraging. Hummingbird territoriality (i.e., "owning" a population of flowers and flying short periods to forage) may be based, at least partly, upon the energetic advantages of using carbohydrate rather than fat. Birds engaging in prolonged activity associated with courtship, mating, or aggressive behavior (and, consequently, having to switch to fat as a fuel) do so at great expense, since they sustain not only the direct energetic costs of such activities but also the indirect costs of a less efficient energy metabolism and a lower rate of net fat deposition as well.

¶Conversion of 4.5 mol of glucose into 1 mol of palmitate yields 5 mol of ATP; subsequent oxidation of 1 mol of palmitate yields 129 mol of ATP, amounting to a net yield of 134 mol of ATP. In contrast, oxidation of 4.5 mol of glucose yields 160 mol of ATP [calculations based on McGilvery (16)].

We thank Mark Roberts and Gordon McIntyre for their generous help throughout the study. This work was funded by Natural Sciences and Engineering Research Council Operating Grants to R.K.S., P.W.H., and C.L.G.

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