Metabolic sources of energy for hummingbird flight

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SUAREZ, RAUL K., GAYLE S. BROWN, AND PETER W. HOCHACHKA. *Metabolic sources of energy for hummingbird flight.* Am. J. Physiol. 251 (Regulatory Integrative Comp. Physiol. 20): R537–R542, 1986.—It has been known for some two decades that hovering flight in hummingbirds is the most energetically expensive muscle work known among vertebrates, but the metabolic support for such work has never been clarified. Measurement of the maximum activities of key enzymes of carbohydrate, fat, and amino acid catabolism in flight muscle and heart of rufous hummingbirds (*Selasphorus rufus*) reveals that the high ATP requirements of short-term hovering flight can only be supported by the oxidation of carbohydrate. Fat oxidation can support a substantially lower maximum rate of ATP turnover, indicating that this process can power only the lower energetic requirements of long-term forward or migratory flight. Mitochondria isolated from flight muscle oxidize pyruvate and palmitoyl-CoA equally well. The inhibition of pyruvate oxidation by palmitoyl-CoA oxidation provides a mechanism by which fat oxidation inhibits carbohydrate oxidation in the transition from short- to long-term flight.

Selasphorus rufus; muscle; energy metabolism; flight energetics; enzymes; mitochondria

HUMMINGBIRDS IN SUSTAINED hovering flight have the highest weight-specific metabolic rates ever recorded among the vertebrates (9, 22). The flight muscles that make this possible, the pectoralis and supracoracoideus, are exceptionally large and make up 21–35% of total body weight (16). This contrasts with other birds in which the flight muscles make up only 10–21% of body weight (12, 16). Both muscles consist exclusively of narrow type I fibers, with giant mitochondria occupying ~50% of total volume (14, 23). These adaptations allow wing-beat frequencies of up to 80/s in the smallest species (13).

Information on the metabolic support for this impressive feat of sustained aerobic performance is limited to the observation of large lipid droplets in the muscle fibers (14, 23), which has led to the proposal that lipid is the major fuel for muscle work (8, 19). Consistent with this interpretation is the observation that certain species are able to fly nonstop across the Gulf of Mexico (a distance of over 800 km) after loading up with ~2 g of fat (over 40% of body wt) (21). Birds, in general, are known to have a high capacity for the storage and utilization of fat (3), which is further augmented before migration (24).

However, there are two problems with this interpretation. First of all, it is widely reported that the highest sustainable muscle work rates are typically supported by carbohydrate, not fat, catabolism (18, 20). Second, since hummingbirds feed mainly on nectar and prefer flowers rich in sucrose, their flight muscles and heart may often have an abundant supply of carbohydrate fuel. Thus, at least under some conditions (e.g., during foraging), hummingbird flight muscles and heart may rely more on carbohydrate as a fuel than may have been appreciated in the past. To gain further insights into these problems, we measured the maximum activities of key enzymes in carbohydrate, fatty acid, and amino acid metabolism. Such an approach allowed a qualitative assessment of the capacity for metabolic flux through these pathways. In addition, the substrate preferences of mitochondria isolated from flight muscle were examined. These data led to reasonably exclusive models for energy metabolism during short- and long-term flight.

MATERIALS AND METHODS

Capture and rearing of birds. Rufous hummingbirds (*Selasphorus rufus*) were captured in May 1985 on the Sechelt Peninsula in southern British Columbia (Canada) and in mid-July in the coastal mountain range of the southern part of the province. Birds caught in the spring, which had recently completed their northward migration were adults in breeding condition. Birds caught in the summer were newly fledged juveniles and adult females that were feeding intensively and accumulating fat stores in preparation for southward migration. All hummingbirds were caught with mist nets or traps and immediately taught to feed from commercially available feeders. They were held in tents overnight before being transported to the laboratory the following day. During transport, birds were restrained individually in small flannel straightjackets to prevent injury and fed a 50% sucrose solution with plastic syringes at 10 to 15-minute intervals.

Birds were kept individually in cages measuring 0.7 × 0.7 × 1.0 m. These were made of wood and soft plastic mesh and contained wooden perches that allowed the birds to rest and feeders which required them to hover to feed. The regular diet consisted of Nektar Plus (Nekton, St. Petersburg, FL) prepared according to the manufacturer's instructions. Fruit flies (*Drosophila*) were supplied twice a week to supplement the diet and to provide additional exercise. During weekends, 50% su-

crose solution replaced the regular diet. Ambient temperature varied between 22 and 27°C. A 14:10 light-dark cycle was maintained, although the holding room was not completely light proof.

Tissue preparation for enzyme assays. Following decapitation, flight muscles were quickly dissected out and cut transversely into sections. No attempt was made to separate the pectoralis from the supracoracoideus because of the small amount obtainable from each bird and because both muscles consist of type I fibers (14, 23). Portions were either immediately processed for determination of enzyme activities or frozen in liquid N_2 and stored at −80°C. Muscle portions were minced with scissors and homogenized with an Ultra-Turrax homogenizer with a 10N shaft. Homogenization was done at 80% of maximum speed for 10–15 s four times at 1-min intervals. Fresh tissue was homogenized in the following at 0–4°C: 50 mM imidazole-Cl (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM dithiothreitol (DTT), 0.1% Triton X-100 (*buffer 1*); 50 mM sodium phosphate (pH 7.4), 2 mM EDTA, 20 mM NaF (*buffer 2*).

Frozen muscle samples were used within 1 wk. Homogenization was conducted as previously described in 50 mM tris(hydroxymethyl)aminomethane chloride (Tris-Cl) (pH 7.6), 1 mM EDTA, and 0.1% Triton X-100 (*buffer 3*). The homogenate was then frozen at −80°C for 30–60 min and thawed out at room temperature. Freeze-thawing was done three times in this manner. Complete warming of the homogenates was avoided; these were refrozen immediately upon thawing while still cold.

Samples homogenized in *buffers 1* and *2* were spun at 17,000 *g* for 5 min at 4°C in a Sorvall RC5C centrifuge. Samples homogenized in *buffer 3* were spun at 12,000 *g* for 2 min in a Micro Centaur microcentrifuge. Supernatant fractions were taken and kept in ice.

Enzyme assays. Enzymes were measured with a Pye-Unicam SP8-400 spectrophotometer with a linear chart recorder and water-jacketed cuvette holders. Assay temperatures were maintained at 39°C with a Lauda K-2/R constant temperature water bath circulator.

Coupling enzymes supplied as precipitates in ammonium sulfate were spun down at 12,000 *g* for 30 s and dissolved in 50 mM imidazole-Cl (pH 7.4), 1 mM EDTA, and 5 mM DTT. Preliminary experiments were conducted to verify saturation of enzymes with substrates and the presence of excess coupling enzymes whenever these were required.

Samples homogenized in *buffer 1* were assayed in 50 mM imidazole-Cl (pH 7.4) under the following conditions. Hexokinase: 5 mM glucose (omitted for control), 1 mM ATP, 5 mM $MgCl_2$, 5 mM DTT, 0.5 mM $NADP^+$, excess glucose-6-phosphate dehydrogenase (G6PDH). Phosphofructokinase (PFK): 10 mM fructose 6-phosphate (omitted for control), 1 mM ATP, 0.15 mM NADH, 2 mM AMP, 10 mM $MgCl_2$, 100 mM KCl, 5 mM DTT, excess aldolase, triose-phosphate isomerase, and α-glycerolphosphate dehydrogenase. Pyruvate kinase (PK): 5 mM phospho(enol)pyruvate (omitted for control), 5 mM ADP, 0.15 mM NADH, 10 mM $MgCl_2$, 100 mM KCl, 0.02 mM fructose 1,6-bisphosphate, 5 mM DTT, excess lactate dehydrogenase. Lactate dehydrogenase (LDH): 4 mM pyruvate (omitted for control), 0.15 mM NADH, 5 mM DTT. Glutamate-oxaloacetate transaminase (GOT): 40 mM aspartate (omitted for control), 10 mM α-ketoglutarate (α-KG), 0.15 mM NADH, 0.025 mM pyridoxal 5-phosphate, 5 mM DTT, excess malate dehydrogenase. Glutamate-pyruvate transaminase (GPT): 200 mM alanine (omitted for control), 10 mM α-KG, 0.15 mM NADH, 0.025 mM pyridoxal 5-phosphate, 5 mM DTT, excess LDH. Glutamate dehydrogenase (GDH): 10 mM α-KG (omitted for control), 1 mM ADP, 100 mM ammonium acetate, 5 mM DTT, 0.15 mM NADH. α-Glycerophosphate dehydrogenase (α-GPDH): 0.4 mM dihydroxyacetone phosphate (omitted for control), 0.15 mM NADH, 5 mM DTT. Samples homogenized in *buffer 2* were assayed in 50 mM sodium phosphate (pH 7.4). Glycogen phosphorylase: 2 mg/ml glycogen (omitted for control), 0.5 mM $NADP^+$, 0.004 mM glucose 1,6-bisphosphate, 2 mM AMP, 10 mM $MgCl_2$, excess phosphoglucomutase, and G6PDH.

Samples homogenized in *buffer 3* were assayed in 50 mM Tris-Cl (pH 8.0) (unless otherwise indicated) as follows. Citrate synthase: 0.5 mM oxaloacetate (omitted for control); 0.3 mM acetyl CoA, 0.1 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). Carnitine palmitoyltransferase (CPT): 5 mM L-carnitine (omitted for control), 0.03 mM palmitoyl-CoA, 0.1 mM DTNB. Malate dehydrogenase (MDH): 50 mM imidazole-Cl (pH 7.4), 10 mM oxaloacetate (omitted for control), 0.15 mM NADH, 5 mM DTT. 3-Hydroxyacyl-CoA dehydrogenase (HOAD): 50 mM imidazole-Cl (pH 7.4), 0.1 mM acetoacetyl CoA (omitted for control), 0.15 mM NADH, 1 mM EDTA, 5 mM DTT. Creatine kinase (CPK): 50 mM imidazole-Cl (pH 7.4), 140 mM creatine phosphate (omitted for control), 1 mM ADP, 20 mM glucose, 1 mM $NADP^+$, 10 mM AMP, 10 mM $MgCl_2$, 5 mM DTT, excess hexokinase, and G6PDH.

Isolation of mitochondria from flight muscle. Muscles from individual birds were quickly dissected out and minced with razor blades in a Petri dish containing cold isolation buffer consisting of 10 mM Tris·Cl (pH 7.4), 210 mM mannitol, 70 mM sucrose, 1 mM ethyleneglycol-bis(β-aminoethyl ether)-*N,N′*-tetraacetic acid (EGTA), and 3 mg/ml fatty acid-free bovine serum albumin. All isolation procedures were done at 0–4°C. The isolation buffer was discarded and muscle fragments were washed with more buffer into a Potter-Elvehjem homogenization vessel. Homogenization was conducted with a loose-fitting Teflon pestle driven by a slow-moving electric drill. The resulting homogenate was spun at 30 *g* for 5 min in a Sorvall RC5C centrifuge. The supernatant fraction was carefully aspirated out and transferred into another centrifuge tube with a Pasteur pipette. This was done without disturbing the pellet. The supernatant was then spun at 1,000 *g* for 5 min. The supernatant fraction from this spin was discarded and the light brown mitochondrial pellet was resuspended in isolation buffer by gentle aspiration with a Pasteur pipette. Care was taken not to disturb the portion of the pellet that consisted of red blood cells. Resuspended mitochondria were then spun

at 1,000 *g* for 5 min. This process of washing was repeated twice, and the pellet from the last centrifugation was resuspended in 0.5 ml of isolation buffer and kept in ice.

Measurement of rates of mitochondrial oxidation. O_2 uptake was monitored with a Gilson oxygraph with a Clark-type O_2 electrode. Incubations were carried out in a final volume of 2 ml in a water-jacketed cell. Temperature was maintained at 39°C with a Lauda K-2/R constant temperature water bath circulator. Cell contents were stirred with a Teflon-covered bar turned by a magnetic stirrer. Mitochondrial suspension and substrates were injected into the cell through a narrow port on top. Assay buffer consisted of 210 mM mannitol, 70 mM sucrose, 10 mM potassium phosphate; 10 mM Tris·Cl (pH 7.4), and 3 mg/ml fatty acid-free bovine serum albumin. Rates of O_2 consumption, respiratory control (RCR), and ADP/O ratios were calculated according to Estabrook (10). Respiratory states are defined according to Chance and Williams (5).

Mitochondrial protein measurement. Mitochondrial protein was measured with a biuret method using 10% deoxycholate to solubilize membrane proteins (11) using bovine serum albumin as the standard.

Measurement of the rate of [1-^{14}C]pyruvate decarboxylation. Mitochondria were incubated in a final volume of 2 ml in glass scintillation vials. The assay buffer was the same as that used for the measurement of rates of O_2 consumption and contained 0.23 μCi[1-^{14}C]pyruvate, 5 mM pyruvate, and 0.1 mM malate. State III conditions were maintained by the addition of 0.2 mM ADP, 10 mM glucose, and 2 units of hexokinase to all of the vials. The vials were covered with rubber serum caps to which plastic center wells were attached. The wells contained 2.4 cm Whatman microfiber filter paper. Temperature was maintained at 39°C with a Blue M water bathshaker. Reactions were initiated by injection of mitochondrial suspension. After shaking for the prescribed amount of time, reactions were terminated by injection of 100 μl of 70% perchloric acid. Controls consisted of flasks into which perchloric acid was injected prior to mitochondria. On termination of reactions, 0.2 ml of Hyamine 10-X hydroxide was injected into the center wells, and the flasks were shaken for 90 min at room temperature. Filter papers were then counted in 5 ml scintillation fluid [100 mg 1,4-bis(5-phenyl-2-oxazolyl)-benzene (POPOP), 2 g 2,5-diphenyloxazole (PPO), 800 ml toluene, and 200 ml ethanol] with a Beckman LS 9000 scintillation counter. Efficiency was ~87% using the method of external standards.

Chemicals. [1-^{14}C]pyruvate was purchased from Amersham. Hyamine 10-X hydroxide and sucrose were from BDH and Schwarz-Mann, respectively. All biochemicals and enzymes were from Sigma and Boehringer-Mannheim. Other chemicals were from various commercial sources and were of reagent grade.

RESULTS

Maximum enzyme activities. Measurements of maximum enzyme activities were conducted at 39°C, which is within the range of pysiological temperatures measured in active hummingbirds (22). This was done to facilitate comparison between capacities for various pathways and metabolic rates calculated from rates of O_2 consumption by intact animals without the need to correct for temperature on the basis of assumed Q_{10} values. Freeze-thawing of the crude homogenate led to a marked increase in citrate synthase activity, indicating increased extraction of mitochondrial protein. Thus activities of this enzyme, as well as those of CPT, MDH, HOAD, and CPK, were routinely measured with freeze-thawed material. Preliminary experiments showed that none of these enzymes was adversely affected by this treatment.

Table 1 lists the activities of 14 enzymes in flight muscle and heart. The highly aerobic nature of both organs is immediately apparent. A high capacity for flux through the Krebs cycle is indicated by extremely high activities of MDH and citrate synthase. The latter enzyme is thought to contribute to regulation of flux through the Krebs cycle and occurs within the range of activities measured in insect flight muscle (1). Citrate synthase activities approaching those found in hummingbirds are reported for pectoral muscles of gray catbirds (24) and American goldfinches (25), whereas lower activities are found in pheasants, sparrows, and pigeons (1). Quite unexpected, based on earlier ideas on the energy metabolism of this tissue (8, 19), is the presence of a high capacity for glycolysis. Higher activities of PK than of LDH and the presence of high hexokinase activities suggest that glycolysis in these organs is designed for high rates of conversion of glycose to pyruvate for complete oxidation of the latter through the Krebs cycle. PK/LDH activity ratios for other vertebrate muscles range between 0.5 and 0.9, whereas in hummingbird flight muscle the PK/LDH ratio is close to 3, indicative of a pathway specialized for carbohydrate oxidation (17).

Tissues capable of high rates of carbohydrate oxidation require a high capacity for transferring reducing equivalents from cytosol to mitochondria. This results from the need to maintain cytosolic redox balance as the glyceraldehyde 3-phosphate dehydrogenase reaction converts NAD^+ to NADH during glycolysis. In vertebrate muscles, cytosolic redox balance is maintained either through the α-glycerophosphate cycle or the malate-aspartate shuttle (26). The presence of high GOT and MDH activities and relatively low α-GPDH activities suggests that the malate-aspartate shuttle is of greater significance in muscle and heart. The absence of measurable amounts of GDH, a mitochondrial matrix enzyme, in these mitochondria-rich organs indicates that amino acids are probably not important oxidative substrates in both organs. CPT activities are high in comparison with values reported for other vertebrate skeletal muscles (7), whereas HOAD activities are similar to those found in gray catbirds (24) and goldfinches (25). These data support the idea that fatty acid oxidation is an important energy source. CPK activities are extremely high in flight muscle and considerably lower in heart. The activities are within the range found in these organs in other species of birds (27). However, the presence of high activities of this enzyme in hummingbird flight muscle appears not to agree with the proposal that higher activities of phosphagen kinases are present in muscles that depend more on anaerobic

TABLE 1. *Maximum enzyme activities in hummingbird flight muscle and heart*

Enzyme	Flight Muscle	*n*	Heart	*n*
Glycogen phosphorylase	31.22±2.5	6	Not measured	
Hexokinase	9.18±0.31	4	10.08±1.9	4
Phosphofructokinase	109.8±13	6	Unstable	
Pyruvate kinase	672.4±27	6	507.3±25	6
Lactate dehydrogenase	230.3±23	6	357.4±30	6
Carnitine palmitoyltransferase	4.42±0.46	6	2.83±0.72	4
3-Hydroxyacyl-CoA dehydrogenase	97.10±13	6	68.51±10	4
Glutamate dehydrogenase	Not detectable		Not detectable	
Glutamate-oxaloacetate transaminase	1,388±70	5	576.4±29	5
Glutamate-pyruvate transaminase	75.97±6.0	5	16.31±2.2	5
Citrate synthase	343.3±8.8	6	190.3±4.8	4
Creatine kinase	2,848±337	5	348.9±59	5
Malate dehydrogenase	3,525±331	6	2,024±191	6
α-Glycerophosphate dehydrogenase	9.37±2.1	6	8.10±2.2	6

Values are expressed in $\mu mol \cdot min^{-1} \cdot g$ wet wt^{-1} and presented as means ± SE. *n*, number of birds.

metabolism than in those that depend on oxidative metabolism for energy production (27), since this tissue is probably the most aerobic vertebrate skeletal muscle known.

Respiration of flight muscle mitochondria. In the course of developing a method for the isolation of mitochondria from flight muscle, it was observed that centrifugation of the crude homogenate at >30 *g* resulted in a supernatant from which only a very small mitochondrial pellet can be obtained by high-speed centrifugation. Thus mitochondria were isolated by spinning the crude homogenate first at 30 *g* and then by spinning the supernatant fraction from this at 1,000 *g*. This is consistent with the observation of giant mitochondria in ultrastructural studies (14, 23).

The ADP dependence of substrate oxidation (state III respiration) and the return to low respiratory rates after depletion of ADP (state IV respiration) (Fig. 1) indicate coupling between respiration and oxidative phosphorylation in preparations of flight muscle mitochondria. The degree of coupling is shown by respiratory control and ADP/O ratios presented in Table 2. Pyruvate and palmitoyl-CoA oxidation are dependent on the presence of malate and support almost equal rates of respiration, a characteristic previously reported for mitochondria from mammalian red skeletal muscle (28). Malate presumably serves as a sparker by augmenting the intramitochondrial oxaloacetate pool since, when supplied alone, it is oxidized at a low rate. Palmitoyl-CoA oxidation is dependent on the presence of L-carnitine, indicating that long-chain fatty acid oxidation occurs via mechanisms similar to those known to operate in mitochondria from mammalian tissues.

Effect of fatty acid oxidation on pyruvate oxidation. The ability of pyruvate and palmitoyl-CoA to support high and almost equal rates of mitochondrial respiration leads to the question of which substrate is preferred when both are available in abundance. Figure 2 shows that 50 μM palmitoyl-L-carnitine causes about a 60% inhibition of [1-^{14}C]pyruvate decarboxylation by mitochondria in state III respiration. This suggests that fatty acid oxidation causes the inhibition of pyruvate dehydrogenase activity in respiring mitochondria, presumably by mechanisms similar to those well studied in mammalian heart

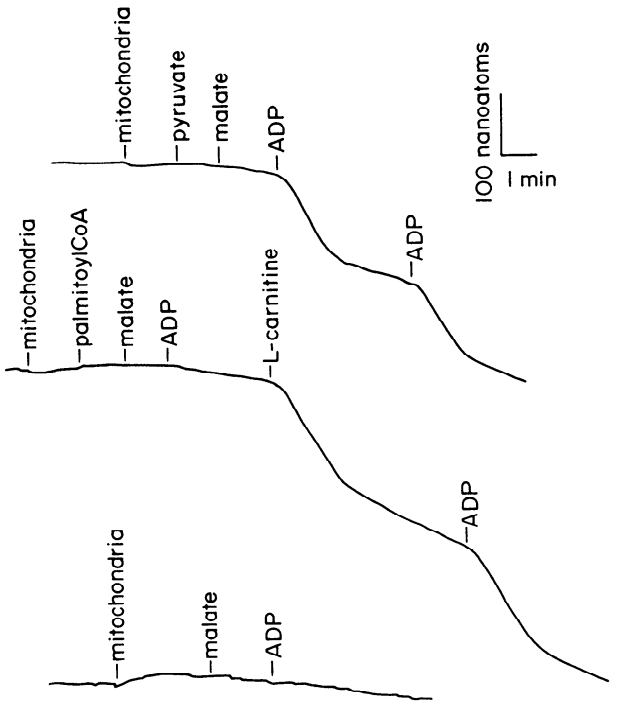

FIG. 1. Substrate oxidation by hummingbird flight muscle mitochondria. Traces result from respiration of 0.33 mg mitochondrial protein. Substrate concentrations are as indicated in Table 2. ADP, adenosine diphosphate; palmitoyl CoA, palmitoyl coenzyme A.

(15). In this way, the oxidation of fatty acid results in inhibition of the oxidation of carbohydrate.

DISCUSSION

Muscle metabolic rate in hovering flight. The most reliable estimates of O_2 consumption by hovering hummingbirds appear to be in the range of 40–50 $ml \cdot g\ bird^{-1} \cdot h^{-1}$ (9, 21, 22, 32). If it is assumed that the flight muscles account for ~30% of total body wt (16) and that this accounts for most of the difference in O_2 uptake between hovering and resting birds (50 $ml \cdot g\ bird^{-1} \cdot h^{-1}$ during exercise − 4 $ml \cdot g\ bird^{-1} \cdot h^{-1}$ at rest = 46 $ml \cdot g\ bird^{-1} \cdot h^{-1}$), then the rate of O_2 consumption per gram of flight muscle is (46 ml $O_2 \cdot g\ bird^{-1} \cdot h^{-1}$)/(0.3 g muscle/g bird) = 153.3 ml $O_2 \cdot g\ muscle^{-1} \cdot h^{-1}$ or ~100.8 $\mu mol\ O_2 \cdot g\ muscle^{-1} \cdot min^{-1}$. Assuming a P/O ratio of 3 or 6 mol of high energy phosphate generated per mole of O_2 con-

TABLE 2. *Substrate oxidation by mitochondria from hummingbird flight muscle*

Substrates	State III Rate	RCR	ADP/O	*n*
First ADP pulse				
5 mM Pyruvate + 0.1 mM malate	318.6±19	5.40±0.46	2.60±0.10	4
0.09 mM Palmitoyl-CoA + 0.1 mM malate + 5 mM L-carnitine	268.0±38	2.70±0.13	2.40±0.09	4
0.1 mM Malate	29.3±3			3
Second ADP pulse				
5 mM Pyruvate + 0.1 mM malate	255.9±12	4.35±0.41	2.80±0.10	4
0.09 mM Palmitoyl-CoA + 0.1 mM malate + 5 mM L-carnitine	276.2±32	2.94±0.19	2.44±0.06	4

Rates are expressed as nanoatoms 0/minute/milligram mitochondrial protein. Data are presented as means ± SE. RCR, (respiratory control ratio) = state III rate/state IV rate; *n*, number of mitochondrial preparations. 0.29–0.38 mg mitochondrial protein and 355 nmol pulses of ADP were injected for assays.

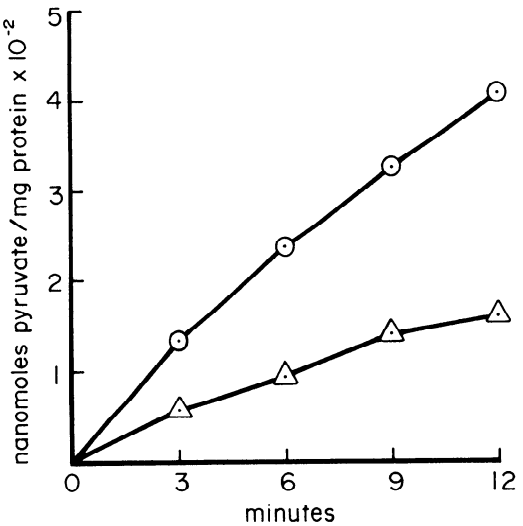

FIG. 2. Effect of palmitoyl L-carnitine on [1-^{14}C]pyruvate decarboxylation by hummingbird flight muscle mitochondria. *Circles*, control; *triangles*, 50 μM palmitoyl L-carnitine.

sumed, this metabolic rate is equivalent to an ATP turnover rate of ~605 μmol·g muscle^{-1}·min^{-1}. Rates of substrate oxidation required to support these ATP turnover rates can be calculated from this value, since 36 mol of ATP are generated per mole of glucose, and 144 mol of ATP are generated per mole of oleate oxidized (26). The latter substrate, rather than palmitate acid, is used for calculation since fatty acid chain lengths of 18 carbons or more account for ~75% of total lipid in these birds (4). If it is assumed that glucose is the sole substrate for hovering flight, a steady-state rate of glucose utilization of 16.8 μmol·g muscle^{-1}·min^{-1} is required. On the other hand, if it is assumed that oleate is the only substrate used, hovering flight would require a rate of oleate oxidation of 4.2 μmol·g muscle^{-1}·min^{-1}.

Potential pathway contributions to muscle work. From the above calculations and our enzyme data (Table 1), a number of informative insights arise. First, the only substrate that, when oxidized exclusively, could sustain muscle work at its highest intensity is endogenous glycogen, since glycogen phosphorylase and all subsequent glycolytic enzyme activities measured in flight muscle exceed the estimated required fluxes by a substantial factor. Second, neither glucose alone nor lipid fuels alone could support the high ATP turnover rates required because of inadequate catalytic capacities of hexokinase and CPT, respectively. Although the hexokinase activities measured are high in comparison with those found in skeletal muscles of other verbetrate species (6), the catalytic capacity of this step is so limited that glucose by itself could at most support only ~50% of the glycolytic flux required to support muscle work during hovering flight. In contrast, the two substrates, glycogen and glucose, taken together could prime a muscle metabolic rate almost threefold higher than the glycolytic flux needed to support muscle during hovering. Although the measured CPT activities are also high in comparison with catalytic capacities found in muscles of other vertebrates (7), they at most could account for only half of the rate of oleate oxidation required for hovering flight, since the two forms of the enzyme, CPT I and CPT II, are situated on the outer and inner surfaces, respectively, of the inner mitochondrial membrane and catalyze different reactions (7).

Such comparisons between metabolic rates calculated on the basis of rates of O_2 consumption by intact animals and maximum possible rates of flux based on enzyme activities must be viewed with caution. The figure we use for mass-specific metabolic rate (50 ml·g bird^{-1}·h^{-1}) has been obtained by three independent groups of investigators and is considered a reasonable estimate for hummingbirds of this size (9, 22, 32). However, it is possible that we have underestimated hexokinase and CPT activities in the present study as a result of inactivation of the enzymes or binding to particulate material. Also, it is not known if hexokinase and CPT are able to function at maximal velocities in vivo; this is generally considered unlikely at these as at most other steps in metabolism (19, 26). Nevertheless, it is clear that the maximal capacity for carbohydrate oxidation exceeds that required to supply the energetic requirements of hovering flight, while it is doubtful whether sufficient capacity for fatty acid oxidation is present in flight muscle for this type of activity. This leads to some interesting biological implications concerning the metabolic support for hovering (short-term) flight and forward and migratory (long-term) flight.

Metabolic costs of short- (hovering) vs. long-term (forward) flight. If our analysis is correct, it is apparent that the preferred fuel for muscle during hovering flight is carbohydrate, although in agreement with previous studies (3, 21), lipid is necessarily the major fuel used for long-term or migratory flight as it is the only fuel stored in adequate abundance. Thus on transition from rest to hovering flight, a powerful activation of glycolysis would be expected. This preference for carbohydrate as a fuel

for hovering flight, generating the highest known rates of ATP turnover in vertebrate muscle, is consistent with glycogen being a better fuel than fat at high rates of ATP turnover in other systems (18, 20). Since the amount of glycogen that can be stored in muscle is limited (19), the extended periods of continuous hovering reported for other species (22) must involve the oxidation of other fuels as well. Extensive simultaneous oxidation of fat and glycogen is considered unlikely because fatty acid oxidation inhibits pyruvate oxidation by isolated flight muscle mitochondria (Fig. 2). Thus it would appear that even if glycogen catabolism alone could power hovering flight for short durations, the degree to which the hovering period is extendable may be strongly dependent on the rate at which glucose can be simultaneously utilized.

For prolonged flight, fat as a fuel has two distinct advantages over glycogen; it can be utilized with greater overall ATP yield, and it can be stored with greater weight efficiency (30). Its known use as the major fuel for long-term migratory flight of hummingbirds means that the power output at this time (or the muscle ATP turnover rate) can only be about half that required in hovering flight. This is consistent with estimates of the relative energetic costs of these kinds of activities (29, 31). The transition from a predominantly carbohydrate-based to a fat-based energy metabolism may occur as a result of a progressive increase in the rate of fatty acid mobilization and delivery to muscle mitochondria. This would result in an increase in the rate of fatty acid oxidation and a concomitant inhibition of carbohydrate oxidation. Such a regulatory interaction seems eminently sensible in view of the large capacity for fat deposition (3, 21) and the limited amount of glycogen that can be stored in muscle (19). An analogous metabolic regulatory system is found in the locust, which flies initially on carbohydrate but switches to fat as the major fuel about 30 min after take off (2).

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REFERENCES

1. ALP, P. R., E. A. NEWSHOLME, AND V. A. ZAMMIT. Activities of citrate synthase and NAD^+-linked and $NADP^+$-linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates. *Biochem. J.* 154: 689–700, 1976.
2. BEENAKKERS, A. M. TH., D. J. VAN DER HORST, AND W. J. A. VAN MARREWIJK. Insect flight muscle metabolism. *Insect Biochem.* 14: 243–260, 1984.
3. BLEM, C. R. Patterns of lipid storage and utilization in birds. *Am. Zool.* 16: 671–684, 1976.
4. CALDWELL, L. D. Fatty acids of migrating birds. *Comp. Biochem. Physiol. B Comp. Biochem.* 44: 493–497, 1973.
5. CHANCE, B., AND G. R. WILLIAMS. The respiratory chain and oxidative phosphorylation. *Adv. Enzymol.* 17: 65–134, 1956.
6. CRABTREE, B., AND E. A. NEWSHOLME. The activities of phosphorylase, hexokinase, phosphofructokinase, lactate dehydrogenase and the glycerol 3-phosphate dehydrogenases in muscles from verbebrates and invertebrates. *Biochem. J.* 126: 49–58, 1972.
7. CRABTREE, B., AND E. A. NEWSHOLME. The activities of lipases and carnitine palmitoyltransferase in muscles from vertebrates and invertebrates. *Biochem. J.* 130: 697–705, 1972.
8. DRUMMOND, G. I. Microenvironment and enzyme function: control of energy metabolism during muscle work. *Am. Zool.* 11: 83–97, 1971.
9. EPTING, R. J. Functional dependence of the power for hovering on wing disc loading in hummingbirds. *Physiol. Zool.* 53: 347–357, 1980.
10. ESTABROOK, R. W. Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. In: *Methods of Enzymology*, edited by S. P. Colowick and N. O. Kaplan. New York: Academic, 1967, vol. 10, p. 41–47.
11. GORNALL, A. G., C. J. BARDAWILL, AND M. M. DAVIE. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177: 751–766, 1949.
12. GREENEWALT, C. H. Dimensional relationships for flying animals. *Smithson. Misc. Collect.* 144: 1–46, 1962.
13. GREENEWALT, C. H. *Hummingbirds.* New York: Doubleday, 1960.
14. GRINYER, I., AND J. C. GEORGE. Some observations on the ultrastructure of the hummingbird pectoral muscles. *Can. J. Zool.* 47: 771–774, 1969.
15. HANSFORD, R. G., AND L. COHEN. Relative importance of pyruvate dehydrogenase interconversion and feed-back inhibition in the effect of fatty acids on pyruvate oxidation by rat heart mitochondria. *Arch. Biochem. Biophys.* 191: 65–81, 1978.
16. HARTMAN, F. A. Locomotor mechanisms of birds. *Smithson. Misc. Collect.* 143: 1–91, 1961.
17. HOCHACHKA, P. W. Exercise limitations at high altitude: the metabolic problem and search for its solution. In: *Circulation, Respiration and Metabolism*, edited by R. Gilles. Berlin: Springer-Verlag, 1985, p. 240–249.
18. HOCHACHKA, P. W., G. P. DOBSON, AND T. P. MOMMSEN. Role of isozymes in metabolic regulation during exercise: insights from comparative studies. *Isozymes Curr. Top. Biol. Med. Res.* 8: 91–113, 1983.
19. HOCHACHKA, P. W., J. R. NEELY, AND W. R. DRIEDZIC. Integration of lipid utilization with Krebs cycle activity in muscle. *Federation Proc.* 36: 2009–2014, 1977.
20. JONES, N. L., G. J. F. HEIGENHAUSER, A. KUKSIS, C. G. MATSOS, J. R. SUTTON AND C. J. TOEWS. Fat metabolism in heavy exercise. *Clin. Sci. Lond.* 59: 469–478, 1980.
21. LASIEWSKI, R. C. The energetics of migrating hummingbirds. *Condor* 64: 324, 1962.
22. LASIEWSKI, R. C. Oxygen consumption of torpid, resting, active and flying hummingbirds. *Physiol. Zool.* 36: 122–140, 1963.
23. LASIEWSKI, R. C., F. R. GALEY, AND C. VASQUEZ. Morphology and physiology of the pectoral muscles of hummingbirds. *Nature Lond.* 205: 404–405, 1965.
24. MARSH, R. L. Catabolic enzyme activities in relation to premigratory fattening and muscle hypertrophy in the gray catbird (*Dumetella carolinensis*). *J. Comp. Physiol.* 141: 417–423, 1981.
25. MARSH, R. L., AND W. R. DAWSON. Substrate metabolism in seasonally acclimatized American goldfinches. *Am. J. Physiol.* 242 (*Regulatory Integrative Comp. Physiol.* 11): R563–R569, 1982.
26. MCGILVERY, R. W. *Biochemistry: A Functional Approach.* Philadelphia, PA: Saunders, 1983.
27. NEWSHOLME, E. A., I. BEIS, A. R. LEECH, AND V. A. ZAMMIT. The role of creatine kinase and arginine kinase in muscle. *Biochem. J.* 172: 533–537, 1978.
28. PANDE, S. V., AND M. C. BLANCHAER. Carbohydrate and fat in energy metabolism of red and white muscle. *Am. J. Physiol.* 220: 549–553, 1971.
29. PENNYCUICK, C. J. Mechanics of bird migration. *Ibis* 111: 525–556, 1969.
30. WEIS-FOGH, T. Metabolism and weight economy in migrating animals, particularly birds and insects. In: *Insects and Physiology*, edited by J. W. L. Beament and J. E. Treherne. New York: American Elsevier, 1968, p. 143–159.
31. WEIS-FOGH, T. Energetics of hovering flight in hummingbirds and *Drosophila*. *J. Exp. Biol.* 56: 79–104, 1972.
32. WOLF, L. L., AND F. R. HAINSWORTH. Time and energy budgets of territorial hummingbirds. *Ecology* 52: 980–988, 1971.