

Fuel Use in Hawkmoth (*Amphion floridensis*) Flight Muscle: Enzyme Activities and Flux Rates

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ABSTRACT The fuels used by the hawkmoth *Amphion floridensis* to power flight are determined by nectar-feeding, with fed moths using primarily carbohydrate and unfed moths using primarily fat. To investigate the metabolic pathways underlying fuel-use flexibility in this species, we measured the maximal activities of several key metabolic enzymes in the flight muscle of fed and unfed individuals, for which metabolic rates and fuel utilization had been previously determined. Hexokinase (HK) and phosphofructokinase (PFK) occur at high activities and, during carbohydrate-fueled flight, are estimated to operate at fractional velocities comparable to those of exclusively carbohydrate-utilizing insects. Females exhibited higher glycolytic enzyme activities than did males, and males regulated PFK activity according to nectar feeding. Although β -hydroxyacyl-CoA dehydrogenase (HOAD) was found at high activities, carnitine palmitoyl transferase (CPT) was not detectable, suggesting that fatty acids may be utilized via a carnitine-independent pathway during flight. Principal component analysis revealed a tendency for the activities of citrate synthase, HK, PFK, and HOAD to be positively correlated among individuals, as well as a lesser tendency for the activities of glycolytic vs. mitochondrial enzymes to be negatively correlated with each other. However, the principal components did not correlate with variation in either oxygen consumption rate or fuel use in vivo, suggesting that variation in enzyme concentration did not determine differences among individuals in metabolic performance during flight. *J. Exp. Zool.* 290:108–114, 2001. © 2001 Wiley-Liss, Inc.

Insect flight metabolism has intrigued researchers for decades. Classic studies by Beenakkers ('69) and Crabtree and Newsholme ('72a,b) characterized metabolic enzyme activities in insect flight muscle to identify the chief metabolic pathways used to power flight. These and many other researchers have found diverse fuel use strategies, with carbohydrates, fatty acids, amino acids, or combinations thereof serving as metabolic substrates in different lineages (reviewed by Candy, '89; Candy et al., '97). A recent study demonstrated that fuel use in a nectar-feeding hawkmoth was facultative: *Amphion floridensis* switched between using carbohydrate or fat as its primary flight fuel, depending on feeding status (O'Brien, '99). Here we assay maximal activities of key enzymes in flight muscle energy metabolism in the same individuals used in the above study to investigate the pathways involved in fuel use in this species and compare activities to rates of energy expenditure in flight. We also test whether activities vary among individual moths according to nectar feeding, sex, and age, and whether they

mirror differences among moths in oxygen consumption rate and metabolic substrate use.

MATERIALS AND METHODS

Moth collecting/rearing

Adult *Amphion floridensis* were trapped using bait (Platt '69) in Princeton, NJ. They were housed in a 0.6 × 0.9 × 1.2 m flight cage, provided potted grape (*Vitis vinifera*) for oviposition, and 30% sugar solution. Eggs were removed from host plant daily. Larvae were reared in 14-cm diameter plastic dishes on freshly collected wild host plant (Family Vitaceae). Adults, eggs, and larvae were kept at 27°C with a 16L:8D photoperiod. Humidity was maintained at 70–80%. Pre-pupae were removed from dishes and allowed to burrow into

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darkened boxes of moist peat moss. *Amphion floridensis* overwinters as pupae; pupae were therefore stored at 4°C. Experimental adults emerged the following summer 10–14 days after being returned to 27°C and 16L:8D photoperiod.

Males and females were housed separately in 0.6 × 0.6 × 0.6 m cages upon emergence. Moths were designated either “Fed” or “Unfed”: fed moths were hand-fed 30% sucrose solution twice daily, whereas unfed moths were treated identically but given water only. Moths were removed either 1 (N = 21) or 5 (N = 18) days after emergence for enzyme analysis. In addition, 12 moths were sampled immediately upon adult emergence.

Respirometry

Oxygen consumption rate was measured and respiratory quotient calculated in 1- and 5-day-old moths using closed-chamber respirometry and sample analysis as reported elsewhere (O'Brien, '99). Briefly, tethered, flying moths were suspended in a 1.7 liter chamber, and kept flying for a period of up to 3 min. Chamber air was sampled initially and after 3 min of flight through a three-way valve, using a 60-cc syringe. Individual moths were sampled in this way at 10-min intervals and were kept flying in between measurements for as long as possible, up to 30 min of flight. Samples were analyzed using an AMETEK S3A single channel oxygen analyzer and a Licor 6251 infrared carbon dioxide analyzer to determine rates of oxygen consumption and carbon dioxide production, respectively.

Enzyme assays

To characterize carbohydrate-oxidizing capacities in the context of long-term, steady-state flight, we assayed hexokinase (HK) and phosphofructokinase (PFK). Glycogen phosphorylase activities were not measured because previous studies indicate that activities of this enzyme are insufficient to account for in-flight flux rates in lepidoptera (Crabtree and Newsholme, '72b). In addition, hawkmoth metabolic rates in flight are so high (O'Brien, '99) that intramuscular glycogen stores would not be expected to support flight for very long. We measured citrate synthase (CS) and β -hydroxyacyl-CoA dehydrogenase (HOAD) to characterize the capacities for Krebs cycle activity and fatty acid metabolism, respectively. In addition, we tested for activities of the mitochondrial enzyme carnitine palmitoyl transferase (CPT), an enzyme involved in carnitine-dependent long-chain fatty acid oxidation.

Homogenization

Following the respirometry measurements, moths were cold anesthetized and the length of the forewing was measured to the nearest 0.1 cm with digital calipers. Moths were weighed and dissected, and their abdomen and thorax were weighed separately (wet weight). Thoraxes were rubbed free of scales and stored whole in liquid nitrogen until homogenization (up to 4 months, with no loss of activity). Upon removal from liquid nitrogen, single thoraxes were chopped coarsely with scissors in nine volumes of ice-cold homogenization buffer (25 mM Hepes, pH 7.5 at 4°C, 2 mM EDTA, 10 mM β -mercaptoethanol (β ME) and 0.1% (vol/vol) Triton X-100), then homogenized in three 10-sec pulses (with 1-min cooling intervals) with a Tissue Tearor homogenizer (Biospec Products, Inc.) at low speed. Homogenates were sonicated in three 10-sec pulses (with 1-min cooling intervals), using a Bronwill Scientific-Biosonix sonicator (Blackstone Ultrasonics) on a power setting of 45.

Homogenates were spun in a refrigerated centrifuge at 2000 rpm for 5 min. The supernatant, including tissue fragments, was removed with a Pasteur pipette and kept on ice for assay. HK and PFK were assayed immediately following homogenization because cytoplasmic enzymes, particularly PFK, tend to be unstable to re-freezing (Henriksson et al., '86). The remaining homogenate was stored in liquid nitrogen for 2–4 weeks until CS and HOAD were assayed. Both of these are mitochondrial enzymes and are stable to freezing and thawing (Suarez et al., '86).

We homogenized thoraxes from four non-experimental moths to test for CPT activity. Because CPT recovery has been shown to be dependent on homogenization conditions (Woeltje et al., '87), we tried homogenization buffers containing different detergents: 0.1% Triton X-100, 1% and 0.1% octyl glucoside, and 1% Tween 20. β ME was omitted from the homogenization buffers to prevent its reaction with DTNB in the assay for CPT activity. Pellets were resuspended in the same volume (i.e., thorax mass × 9) of the buffers used for tissue homogenization and also assayed for activity.

Assay protocols

All enzyme activities were measured in 1-ml cuvettes at 37°C in a Beckman DU64 spectrophotometer, with a water-jacketed six-cell cuvette changer. This temperature represents a typical in-flight thoracic temperature for hawkmoths (Heinrich, '71). NADH- or NADPH-linked assays were

monitored at 340 nm; while dithiobisnitrobenzoic acid (DTNB)-linked reactions were monitored at 412 nm. Concentrations of substrates, cofactors, and coupling enzymes were varied the day of the assay to ensure that the catalytic rates measured were maximal. Enzymes were purchased from either Sigma Chemical (St. Louis, MO) or Boehringer Mannheim (Indianapolis, IN), and all other chemicals were purchased from Sigma.

Cytoplasmic enzymes

Hexokinase (HK) was assayed with 5 mM glucose (omitted for control), 10 mM ATP, 10 mM $MgCl_2$, 100 mM KCl, 0.5 mM NADP, 10 mM β ME, and excess glucose-6-phosphate dehydrogenase in 50 mM Hepes buffer (pH 7 at 37°C). Phosphofructokinase (PFK) was assayed with 5 mM fructose-6-phosphate (omitted for control), 10 mM ATP, 0.15 mM NADH, 0.01 mM fructose-2,6-bisphosphate, 10 mM $MgCl_2$, 100 mM KCl, 10 mM β ME, and excess aldolase, triose phosphate isomerase, and α -glycerophosphate dehydrogenase in 50 mM TrisCl buffer, (pH 8.0 at 37°C). PFK is destabilized at pH 7.0 in vitro, therefore, it is assayed at pH 8.0 (Suarez et al., '96; Wegener et al., '87). ATP had an inhibitory effect only above 20 mM in both HK and PFK assays. Addition of an ATP regeneration system using arginine kinase and arginine phosphate had no effect on reaction rates for either HK or PFK.

Mitochondrial enzymes

All enzymes were assayed in 50 mM TrisCl (pH 8.0 at 37°C). β -hydroxyacyl-CoA dehydrogenase (HOAD) was assayed with 0.2 mM acetoacetyl CoA (omitted for control), 0.15 mM NADH, 1 mM EDTA, and 10 mM β ME. Carnitine palmitoyl transferase (CPT) was assayed with 5 mM L-carnitine (omitted for control), 0.03 mM palmitoyl CoA, and 0.1 mM DTNB. Citrate synthase (CS) was assayed with 0.5 mM oxaloacetate (omitted for control), 0.3 mM acetyl CoA, and 0.1 mM DTNB. Because HOAD and CS activities were high, homogenates were diluted 1:20 to yield a longer linear phase of reaction. We found that diluting homogenate with β ME-free homogenization buffer made DTNB-linked CS assays possible without interference by β ME.

Statistical analyses

All statistical analyses were performed using JMP version 3.1 (SAS Institute). Means are reported \pm SE. We tested for the effects of feeding treatment, sex, and age on the activity of each

enzyme using a full factorial ANOVA, then removed terms that were not significant. We performed ANCOVA with the remaining factors, testing body mass, oxygen consumption rate, and respiratory quotient as covariates. The final models (from which F and P are reported) contained significant factors and covariates only. All other statistical tests are noted in the text.

RESULTS

Maximal enzyme activities are presented in Table 1 for both freshly emerged moths and experimental moths. Vmax data are presented both as means across all samples and means within categories of sex, age, and feeding treatment (Table 1). Activity increased after adult emergence for all enzymes except hexokinase ($P < 0.02$, one-way ANOVA).

v/Vmax

Average oxygen consumption rate standardized by thoracic mass for experimental moths (moths for which we also have respirometry data) was 257.3 ± 13.5 ml O_2 hr^{-1} g thorax $^{-1}$ (mean \pm SE, N = 31) (O'Brien, '99). This oxygen consumption rate expressed at STP was 182.3 ml O_2 hr^{-1} g thorax $^{-1}$, and was converted to μ mol O_2 per minute using 22.4 l/mol (= 44.6 μ mol/ml): 182.3 ml O_2 hr^{-1} g thorax $^{-1} \cdot 1$ hr/60 min $\cdot 44.6$ μ mol O_2 /ml O_2 = 135.5 μ mol O_2 min $^{-1}$ g thorax $^{-1}$.

Because we are interested in determining whether these oxygen flux rates could be supported by carbohydrate alone, we calculate the ratio of enzyme activity to flux rate assuming that carbohydrates are used exclusively. The oxygen flux rate can be converted to the glycolytic flux rate (v) according to the following calculation: 135.5 μ mol O_2 min $^{-1}$ g thorax $^{-1} \div 6$ O_2 /glucose = 22.6 μ mol substrate min $^{-1}$ g thorax $^{-1}$.

The flux rates through HK and PFK would be equal to this value, assuming steady-state flux through glycolysis during flight fueled by hemolymph-borne carbohydrates. Because carbohydrate fuels the majority of flight expense in fed moths over flights up to 30 min in duration (O'Brien, '99), the assumption of steady state glycolytic flux is well justified. The glycolytic flux rate divided by enzyme maximal activities yield fractional velocities, v/Vmax, at the HK and PFK steps of 0.64 and 0.32, respectively.

Correlations among enzyme activities

We performed principal components analysis to clarify patterns of variation within our enzyme ac-

TABLE 1. Flight muscle maximal enzyme activities (V_{max})¹ for freshly eclosed and experimental² moths

Enzyme [Pathway]	Sex	Freshly eclosed	Day 1		Day 5		Mean (all)
			Fed	Unfed	Fed	Unfed	
Hexokinase (HK) [Glycolysis]	M	33.1 ± 2.5 (6)	29.1 ± 1.7 (6)	32.7 ± 1.9 (4)	35.4 ± 3.1 (4)	33.3 ± 3.6 (4)	34.3 ± 0.7 (51)
	F	34.4 ± 2.5 (6)	35.8 ± 1.4 (6)	37.5 ± 0.5 (5)	35.9 ± 1.2 (5)	36.8 ± 2.0 (5)	
Phosphofructokinase (PFK) [Glycolysis]	M	56.0 ± 3.2 (6)	63.7 ± 2.1 (6)	56.4 ± 3.3 (4)	74.2 ± 2.3 (4)	63.2 ± 1.6 (4)	67.5 ± 1.3 (51)
	F	65.9 ± 4.6 (6)	74.5 ± 2.1 (6)	76.9 ± 2.4 (5)	70.8 ± 2.4 (5)	73.2 ± 2.8 (5)	
β -Hydroxyacyl-CoA Dehydrogenase (HOAD) [β -Oxidation]	M	142.8 ± 14.0 (6)	154.6 ± 15.9 (6)	155.9 ± 7.6 (4)	147.1 ± 9.9 (4)	188.5 ± 21.0 (4)	159.8 ± 4.9 (50)
	F	138.3 ± 15.9 (6)	187.8 ± 15.7 (5)	168.5 ± 14.0 (5)	162.1 ± 17.4 (5)	163.7 ± 9.0 (5)	
Citrate Synthase (CS) [Krebs Cycle]	M	410.8 ± 34.6 (6)	458.1 ± 23.0 (6)	497.1 ± 10.2 (4)	546.0 ± 7.5 (4)	549.0 ± 30.3 (4)	476.5 ± 11.1 (51)
	F	373.6 ± 28.4 (6)	504.2 ± 43.9 (6)	480.2 ± 23.6 (5)	510.3 ± 20.0 (5)	500.4 ± 14.0 (5)	

¹ V_{max} is expressed as $\mu\text{mol substrate g thorax}^{-1} \text{min}^{-1}$. Shown are means \pm Standard Error (sample size in parentheses).

²Experimental moths were either fed or unfed for 1 or 5 days.

tivity data set (Sabat et al., '98; Berrigan and Hoang, '99). Principal components analysis is a multivariate statistical technique that reduces a number of correlated variables (here, enzyme activities) into linear combinations that are statistically independent of each other. These combinations, termed principal components, summarize patterns of correlation and can be interpreted fairly intuitively (Sokal and Rohlf, '95). The first two principal components, which explain 75% of the total variation in enzyme activity, are presented in Table 2. The first principal component (PC1) was positively correlated with activities of all of the enzymes, indicating a tendency for individuals to have higher or lower activities of all four enzymes in parallel, and explained 49% of the total variation in activity. The second principal component (PC2) explained 26% of the variation in enzyme activities, and was positively correlated with activities of the mitochondrial en-

zymes HOAD and CS, but negatively correlated with both glycolytic enzymes (HK and PFK). PC2 thus indicates that a tendency for individuals with higher glycolytic enzyme activities to have lower mitochondrial enzyme activities, and vice versa. Neither PC1 nor PC2 were significantly correlated with body mass, oxygen consumption rate or respiratory quotient. Nor did body mass, oxygen consumption rate, or respiratory quotient significantly co-vary with activities of any individual enzyme (reported below).

Effects of feeding treatment, sex, and age on glycolytic enzymes

Activities of both HK and PFK were significantly higher (~15%) in females than in males (HK: $F_{1,38} = 14.97$, $P = 0.0005$, PFK: Table 3, Fig. 1). PFK activity increased by about 15% with both sucrose feeding and with age in male moths (significant sex \times feeding treatment and sex \times day interaction terms, Table 3, Fig. 1).

Effects of feeding treatment, sex and age on mitochondrial enzymes

CS activity increased with age ($F_{1,38} = 8.6$, $P = 0.0061$), but not feeding or sex, whereas HOAD activity was not affected by any of the factors.

Carnitine palmitoyl-transferase

CPT activity was not detectable with the methods described here. We verified the assay protocol by measuring CPT activity in frozen mackerel heart tissue and measured an activity of 5.6 μmol

TABLE 2. Results of principal components analysis of the activities of metabolic enzymes from *Amphion floridensis* flight muscle ($N=39$)

	PC1	PC2
Percent variation explained	48.7	25.9
Cumulative percent	48.7	74.6
Enzymes:		
Hexokinase	0.529 ¹	-0.434
Phosphofructo-kinase	0.450	-0.520
β -Hydroxyacyl-CoA Dehydrogenase	0.428	0.668
Citrate synthase	0.578	0.307

Only the first two principal components axis are presented.

¹These values indicate the strength and direction of the correlations between the principal component and each enzyme activity.

TABLE 3. ANOVA demonstrating the effects of feeding treatment, sex and age on PFK activity.

Effect	SS	df	F Ratio	Significance ¹
Sex	810.5	1	30.3	<0.0001
Treatment	87.6	1	3.3	0.0799
Sex × treatment	332.5	1	12.4	0.0013
Day	74.2	1	2.8	0.1058
Sex × day	337.2	1	12.6	0.0012
Error	856.8	32		

Only significant interaction terms are presented (N=39)

¹Significant effects are in bold.

$\text{g}^{-1} \text{min}^{-1}$. Moths supporting flight with fatty acid oxidation would oxidize palmitate at a rate of $135.5 \mu\text{mol O}_2 \text{min}^{-1} \text{g thorax}^{-1} \div 23 \text{ O}_2 \text{ palmitate}^{-1} = 5.98 \mu\text{mol palmitate g thorax}^{-1} \text{min}^{-1}$. Therefore, sufficient enzyme activity should have been present to be detected by our method (Suarez et al., '86).

Mass proportioning

Because females had higher flight muscle activities of HK and PFK than males, we investigated whether there were differences in male and female morphology that might affect energy demands in flight. Previous analyses have shown that females were 30% heavier than males at emergence; however, those mass differences were not sufficient to significantly affect either metabolic rate or respiratory quotient (O'Brien, '99). Here we test whether mass proportioning (abdomen mass/thorax mass) or wingloading (estimated

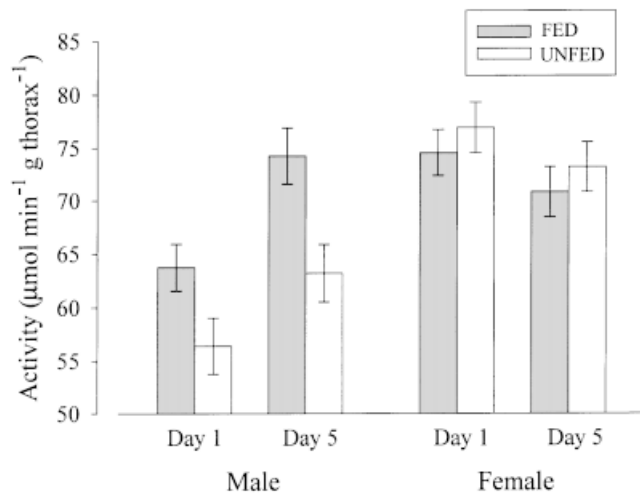


Fig. 1. The effect of feeding treatment and age on PFK activity in male and female moths. Filled bars denote fed moths, whereas open bars denote unfed moths. Both age and feeding significantly affect PFK activity in males, whereas neither affects PFK activity in females. Shown are least squares means \pm SE; total N = 39.

as body mass/wing length) differ among sexes, ages, and feeding treatments (Table 4, ANOVA, only significant effects reported), and whether those differences affect flight metabolism. Females had significantly heavier abdomens per gram thorax (~ flight muscle) than males, and heavier bodies relative to wing length (Table 4). Feeding treatment had an even slightly larger effect; with fed moths having heavier abdomens and bodies relative to thoracic mass and wing length, respectively, than unfed moths (Table 4). However, neither ratio bore a significant relationship to oxygen consumption rate or metabolic substrate use in flight.

DISCUSSION

Activities of key metabolic enzymes in *Amphion* flight muscle support in vivo measurements suggesting that these hawkmoths can easily use either carbohydrates or fatty acids to fuel the high metabolic rates required during flight (O'Brien, '99). Glycolytic enzyme activities in *Amphion* are close to those of the exclusively carbohydrate-utilizing flight muscles of honeybees (Suarez et al., '96). When moths rely on dietary carbohydrates alone to fuel long-term flight, the fractional velocities at which HK and PFK operate in vivo would be similar to those estimated in flying honeybees (Suarez et al., '96). The fractional velocities at these steps fall at the high end of the range of v/V_{max} values measured in exercising animals (Suarez et al., '97; Suarez, '98).

Activities of CS and HOAD are also very high, as would be expected from the high rates of aerobic metabolism during flight and the ability to rely exclusively on fatty acid oxidation to power flight (Casey, '76; Bartholomew and Casey, '78; O'Brien, '99). However, we do not directly compare HOAD and CS activities to physiological flux rates. Considerable evidence suggests that both the β -oxidation spiral and the Krebs cycle are channeled pathways, in which enzymes operate within a multi-enzyme complex and metabolic intermediates do not go into solution (Srere, '80; Srere and Sumegi, '94). Thus, as in previous studies (e.g., Suarez et al., '90, '96), we do not attach any mechanistic significance to v/V_{max} values at these steps.

The activities of HK, PFK, CS, and HOAD tended to be positively correlated (PC1), suggesting differences in overall metabolic capability among individuals. A weaker, negative relationship between the activities of glycolytic and mitochondrial enzymes (PC2) suggests that individuals could be biased toward greater or lesser degrees

TABLE 4. Effects of sex and feeding treatment on the proportioning of body mass

Dependent variable	SEX				TREATMENT			
	Female	Male	F	P	Fed	Unfed	F	P
Abd. mass/total mass	0.590	0.501	36.63	<0.0001	0.594	0.498	42.24	<0.0001
Abd. mass/thx. mass	2.44	1.67	31.64	<0.0001	2.50	1.60	43.57	<0.0001
Thx. mass/abd mass	0.453	0.643	24.76	<0.0001	0.428	0.669	39.63	<0.0001
Total mass/wing length ¹	0.022	0.017	14.71	<0.0009	0.023	0.016	20.18	<0.0002

N = 39 except where noted.

¹N = 29.

of carbohydrate utilization. However, neither trend was correlated with either oxygen consumption rate or with metabolic substrate use (as indicated by the respiratory quotient) in vivo. There were also no relationships between the activity of any enzyme tested individually and either oxygen consumption rate or metabolic substrate use. These findings are consistent with the results obtained in other studies. For example, in work with a wide array of metabolic enzymes in *Drosophila*, Laurie Ahlberg and colleagues identified significant variation in both enzyme activity (Laurie Ahlberg et al., '80; Laurie Ahlberg et al., '82) and flight performance (Curtsinger and Laurie Ahlberg, '81). Despite this variation, correlations between activity and performance were generally weak where they existed at all (Laurie Ahlberg et al., '85). Their data and our own suggest that variation in enzyme activity among individuals, even when statistically significant, may not be of sufficient magnitude to cause tangible effects on whole animal metabolic rate in vivo. An important caveat, however, is that a flying animal's oxygen consumption rates are underestimated in enclosed respirometry chambers (Rayner and Thomas, '91). It is possible that a relationship between oxygen consumption rate and enzyme activities could be expressed in animals that are exercising maximally in natural conditions.

Males and females clearly differed in their regulation of glycolytic enzyme activity. Females had approximately 15% greater activity of both HK and PFK than did males. Interestingly, this result contrasts a tendency of females to (on average) utilize less carbohydrate in flight, while exhibiting identical rates of oxygen consumption (O'Brien, '99). Differences in mass proportioning between males and females had no effect on in-flight oxygen consumption or fuel selection. A possible explanation may be differences in nutrient requirements related to reproduction. The carbon skeletons of nonessential amino acids in eggs are synthesized primarily from dietary carbohydrates,

which results in high rates of carbohydrate turnover in females (O'Brien et al., 2000, O'Brien, D.M., Fogel, M.L, and Boggs, C.L., unpublished). It is possible that systemically higher activities of glycolytic enzyme in females may be related to these demands; however, this hypothesis remains to be addressed empirically.

Although males generally exhibited lower activities of glycolytic enzyme than females, PFK activity increased in males within hours of first nectar feeding, and achieved the levels found in females after 5 days. When fed, males rely on carbohydrates to a greater degree in flight than do females (O'Brien, '99). The rapid increase in PFK activity after feeding may compensate for lower levels of glycolytic enzyme in unfed males. Why HK did not show a similar increase, however, is unclear.

The apparent absence of carnitine palmitoyl transferase (CPT) in *Amphion* flight muscles is intriguing. Two possible explanations are apparent: First, the two forms of the enzyme (CPT1 and CPT2) could have unusual properties in hawkmoth flight muscle that resulted in their inactivation during extraction or assay. This seems unlikely because versions of our methods have been successfully used to assay CPT in a wide variety of organisms including yeast, molluscs, insects, and vertebrates (Rodnick and Sidell, '94; Stuart and Ballantyne, '96; Joannis and Storey, '96; De Vries et al., '97). An alternative explanation is that *Amphion* is capable of carnitine-independent fatty acid oxidation. This suggestion is supported by a previous report of carnitine-independent fatty acid oxidation in the flight muscle mitochondria of the noctuid moths *Prodenia eridania* and *Trichoplusia ni* (Stevenson, '68). However, Hansford and Johnson ('76) reported carnitine-dependent fatty acid oxidation in the hawkmoth *Manduca sexta*, indicating that CPT1 and CPT2 assume their well-established roles in this species. These findings raise the possibility of interspecific differences in the pathways of long chain fatty acid oxidation in moth flight muscles.

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