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Seasonal upregulation of catabolic enzymes and fatty acid transporters in the flight muscle of migrating hoary bats, *Lasiurus cinereus*

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ABSTRACT

The high energy density of fat, and limited capacity for carbohydrate storage suggest that migrating bats should fuel endurance flights with fat, as observed in migrating birds. Yet, cursorial mammals are unable to support high intensity exercise with fat stores. We hypothesized that migratory bats and birds have converged on similar physiological mechanisms to fuel endurance flight with fat. We predicted bats would seasonally upregulate fatty acid transport and oxidation pathways when migration demands were high. We studied seasonal variation in mitochondrial oxidative enzyme activities and fatty acid transport protein expression in the flight muscle of hoary bats (*Lasiurus cinereus*). Carnitine palmitoyl transferase, 3-hydroxyacyl-CoA dehydrogenase and citrate synthase activity increased during migration. There were no changes in expression of fatty acid translocase or plasma membrane fatty acid binding protein. Heart-type fatty acid binding protein expression increased 5-fold in migrating females, but did not vary seasonally in males. An aerial insectivore lifestyle, and the coincidence of migration and pregnancy may explain differences in transporter expression compared to previously studied birds. Overall, our results are consistent with seasonal upregulation of lipid metabolism and aerobic capacity, and confirm that migration poses distinct physiological challenges for bats.

1. Introduction

Flying bats expend energy at about 15 times basal metabolic rate, and there is no difference in the cost of flight between bats and birds (Speakman and Thomas, 2003). Migrating bats must maintain this high level of expenditure for several hours during migratory flights. Foraging bats are able to fuel extended periods of flight with recently ingested nutrients (Welch et al., 2008; Voigt et al., 2010), and although migrating bats may feed regularly during migration (Valdez and Cryan, 2009; Reimer et al., 2010; Voigt et al., 2012; McGuire et al., 2013), recent evidence suggests that bats rely on stored nutrients to fuel extended migratory flights (Voigt et al., 2012). According to the mammalian exercise model (McClelland, 2004), mammals typically fuel high intensity exercise with carbohydrates. In the fasted, flying state of a migrating bat, glycogen stores would be quickly depleted (Yacoe et al., 1982). Therefore the flight muscles of migrating bats, like those of birds, should use extra-muscular fat stores as their main source of fuel (Jenni and Jenni-Eiermann, 1998; McGuire and Guglielmo, 2009).

Using extra-muscular fat stores as fuel for flight has been shown in birds to require enhanced pathways for fatty acid transport and oxidation (Guglielmo, 2010). Many studies of flight muscle metabolism in migratory birds have documented increases in oxidative enzyme activities during migratory periods (Marsh, 1981; Lundgren and Kiessling, 1985; Driedzic et al., 1993; Guglielmo et al., 2002; McFarlan et al., 2009). In birds, three enzymes in particular are seasonally modulated: carnitine palmitoyl transferase (CPT; EC 2.3.1.21), 3-hydroxyacyl-CoA dehydrogenase (HOAD; EC 1.1.1.35), and citrate synthase (CS; EC 2.3.3.1). CPT is involved in translocating fatty acids from the cytosol across the mitochondrial membrane (Suarez et al., 2009). HOAD is an enzyme in the β-oxidation pathway which provides an index of fatty acid catabolism. CS is an enzyme of the Kreb's cycle and is therefore indicative of muscle aerobic capacity. Studies of oxidative enzymes in bat flight muscle suggest a high aerobic capacity (Armstrong et al., 1977; Yacoe et al., 1982; Suarez et al., 2009) and fatty acid oxidation potential (Yacoe et al., 1982; Suarez et al., 2009). Comparisons of active and hibernating bats have demonstrated seasonal variation in enzyme activity (Armstrong et al., 1977; Yacoe, 1983; Brigham et al., 1990; Kim et al., 2000), but seasonal variation associated with migration has not been considered in bats.

The capacity to transport fatty acids into muscles is an important factor in the maintenance of high rates of oxidative metabolism during migratory flight in birds (Guglielmo et al., 1998; Pelsers et al., 1999; Guglielmo et al., 2002; McFarlan et al., 2009; Price et al., 2010). Due to the low solubility of lipids, each step in the transport pathway from adipose stores to muscle mitochondria must be mediated by protein transporters (Guglielmo, 2010). Currently, migratory birds are thought to maintain high metabolic rates for long periods of time due to high

Abbreviations: CPT, carnitine palmitoyl transferase; HOAD, 3-hydroxyacyl-CoA dehydrogenase; CS, citrate synthase; FABPpm, plasma-membrane fatty acid binding protein; FAT/CD36, fatty acid translocase; H-FABP, heart-type fatty acid binding protein.

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levels of fatty acid transport protein expression in flight muscles (McFarlan et al., 2009). Transport of fatty acids into muscle cells is achieved primarily by the action of two membrane-bound fatty acid transport proteins: plasma-membrane fatty acid binding protein (FABPpm) and fatty acid translocase (FAT/CD36) (Bonen et al., 2007). Inhibition studies have demonstrated that FAT/CD36 may be responsible for up to 50% of protein mediated fatty acid transport in muscle cells (Luiken et al., 1999). Furthermore, FAT/CD36 may act synergistically with FABPpm to increase fatty acid transport rates (Bonen et al., 2007). FAT/CD36 and FABPpm are seasonally up-regulated in migrating white-throated sparrows (*Zonotrichia albicollis*) with 70–150% relative increases in mRNA expression (McFarlan et al., 2009). Within the muscle cell cytosol, fatty acids are transported from the cell membrane to the mitochondria by heart-type fatty acid binding protein (H-FABP). A large pool of H-FABP may act as a fatty acid sink, maintaining high rates of fatty acid flux across the cell membrane (Bonen et al., 2007). H-FABP is seasonally up-regulated during migration, with increased protein concentration observed in barnacle geese (*Branta leucopsis*) (Pelsers et al., 1999), western sandpipers (*Calidris mauri*) (Guglielmo et al., 2002) and white-throated sparrows (McFarlan et al., 2009). In white-throated sparrows, relative mRNA expression in migrating birds was approximately 11 times greater than non-migrating birds (McFarlan et al., 2009). The role of fatty acid transporters in bat migration has not been studied. However, H-FABP is up-regulated in hibernating bats (Eddy and Storey, 2004), a period when the bats rely exclusively on stored fat, suggesting that fatty acid transporters may be up-regulated in migration.

We investigated whether seasonal physiological changes in flight muscle biochemistry that have been observed in migratory birds would be evident in a migratory bat. We studied seasonal changes in mitochondrial catabolic enzyme activity and flight muscle fatty acid transport protein expression in the long-distance latitudinal migrant hoary bat (*Lasiurus cinereus*). We predicted migrating bats would increase CPT, HOAD, and CS activities, and mRNA expression of H-FABP, FAT/CD36, and FABPpm in flight muscles.

2. Materials and methods

2.1. Animal collection

The hoary bat (*L. cinereus*) is a ~20–35 g insectivore in the family Vespertilionidae. It is the most widely distributed bat species in North America, breeding as far north as the boreal forest of Canada (Shump and Shump, 1982). The winter distribution is poorly documented, but it is thought that hoary bats overwinter in Mexico and southern California (Cryan, 2003). Based on stable isotope analysis, some individuals are thought to migrate >2000 km between summer and winter grounds (Cryan et al., 2004). See Cryan (2003) for a more detailed description of the annual distribution patterns of the species, including differences in male and female summer distribution resulting from sex-biased differential migration patterns (see Section 4 below).

We captured adult, non-migrating bats during the summer residency period, after pups had been weaned but before the onset of autumn migration. We collected bats from 20 July to 1 August, 2008 and 2009 in mist nets set across creeks in Cypress Hills Interprovincial Park, Saskatchewan, Canada (49° 34′ N, 109° 53′ W). We identified sub-adult bats by the degree of ossification of the metacarpal–phalanges joint (Anthony, 1988), and lactating females were identified by manually expressing milk from the mammary glands. All sub-adults and lactating females were released immediately.

We collected migrating hoary bats as they moved north through New Mexico in the spring. We collected bats from May 5 to 17, 2009 by setting mist nets over creeks and small ponds in Bernalillo County, New Mexico, USA (35° 12′ N, 106° 18′ W) and in the Manzano Mountains, Cibola National Forest, New Mexico, USA (34° 59′ N, 106° 21′ W).

In Saskatchewan we collected 8 female and 7 male non-migrants, and in New Mexico we collected 15 female and 15 male migrants. Immediately upon capture we euthanized the bats by cervical dislocation under isoflurane anesthesia. We recorded body mass (±0.1 g) and forearm length (±0.05 mm) and quickly dissected a sample of pectoralis muscle. The pectoralis sample was transferred to a 2 mL cryotube (Cryo.S, Grenier Bio-one) and frozen in a liquid nitrogen cooled cryoshipper (Taylor-Wharton CX-100). The samples were transported back to the lab either in the liquid nitrogen cooled cryoshipper or packed in dry ice, and stored at −80 °C until analysis.

All animal collection and experimental protocols were approved by the University of Western Ontario Animal Use Sub-committee (protocol no. 2008-003-04) and conducted under permits from the New Mexico Department of Game and Fish (permit no. 3424), United States Department of Agriculture — Forest Service (permit no. SND502), Saskatchewan Ministry of Environment (permit nos. 08FW080 & 09FW045), and Saskatchewan Ministry of Tourism, Parks, Culture and Sport (permit nos. SP-CHPP-02-08 & SP-CHPP-01-09). Samples from New Mexico were imported to Canada under the approval of the Canadian Food Inspection Agency (permit no. A-2009-01022-3).

2.2. Enzyme assays

Tissue preparation and enzyme assays were adapted from methods described in Price et al. (2010). Approximately 100 mg of pectoralis muscle was combined with 9 volumes of homogenization buffer (20 mM Na_2HPO_4, 0.5 mM EDTA, 0.2% defatted BSA, 50% glycerol, 0.1% Triton X-100, and 50 μg/mL aprotinin). Keeping the sample on ice, we homogenized 3 × 10 s (Polytron PT 10-35, Kinematica Inc., Bohemia, NY, USA), allowing the sample to rest for 30 s between bouts. We set the speed of the homogenizer to the highest setting which did not result in foaming. Homogenates were sonicated 3 × 10 s, waiting 30 s on ice between bouts, then stored at −80 °C until analysis.

All enzyme assays were performed at 39 °C with a 1 mL reaction volume on a Cary 100 Bio Spectrophotometer (Varian, Palo Alto, CA, USA). CPT was assayed in 50 mM Tris buffer (pH 8.0) with 2.5 mM carnitine, 0.15 mM DTNB, 8.75 μM palmitoyl CoA and 10 μL homogenate diluted 1:5. HOAD was assayed in 50 mM imidazole buffer (pH 7.4) with 2 mM EDTA, 0.2 mM NADH, 0.2 mM acetoacetyl CoA, and 10 μL homogenate diluted 1:10. CS was assayed in 50 mM Tris buffer (pH 8.0) with 0.6 mM acetyl CoA, 0.15 mM DTNB, 0.5 mM oxaloacetic acid, and 10 μL homogenate diluted 1:10. CS and CPT activities were calculated from ΔA_{412} and HOAD activity was calculated from ΔA_{340}.

2.3. Transporter mRNA expression

2.3.1. RNA isolation and reverse transcription

RNA isolation and reverse transcription followed the methods of Price et al. (2010). We isolated total RNA by extracting 50–100 mg of frozen muscle tissue in 1 mL TRIzol reagent following the manufacturer's protocol (Invitrogen, Burlington, ON, Canada) with two additional chloroform extractions and ethanol washes. The RNA pellet was stored at −20 °C until analysis. RNA samples were eluted in sterile water and dissolved by heating to 55 °C. To quantify the concentration and quality of the RNA, we measured absorbance (in Tris-EDTA buffer — 10 mmol L^{-1} Tris, pH 8, 1 mmol L^{-1} EDTA) at 260 nm and 280 nm. All samples were of high quality as indicated by A_{260}:A_{280} > 1.8. DNA contamination was removed by incubating 5 μg RNA with 2 i.u. DNase I (New England Biolabs) at 37 °C for 15 min and inactivating at 75 °C for 10 min. We reverse transcribed RNA to cDNA with 0.5 μg RNA, 0.5 μg oligo-$dT_{12–18}$ primer, 0.5 mM of each dNTP, 1 × FS buffer, 10 mM DTT, 1 μL RNase OUT, and 200 U SuperScript II reverse transcriptase in 20 μL total reaction volume (all reagents from Invitrogen). Samples were incubated for 90 min at 42 °C and then 10 min at 70 °C to stop the reaction. cDNA samples were stored at −80 °C until analysis.