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BROWN FAT IN BIRDS? A TEST FOR THE MAMMALIAN BAT-SPECIFIC MITOCHONDRIAL UNCOUPLING PROTEIN IN COMMON POORWILLS[1]

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In mammals, brown adipose tissue (BAT) or brown fat is the major organ of thermoregulatory heat production through non-shivering thermogenesis (NST; Nicholls and Locke 1984). It is commonly found in neonates, cold-adapted adults, and hibernators (Rothwell and Stock 1985). Heat is generated through a mitochondrial proton conductance pathway which uncouples substrate oxidation from ATP synthesis. This process is regulated by a 32,000–33,000 M_r "uncoupling protein" (Nicholls and Locke 1984, Nicholls et al. 1986) that is specific to brown adipose tissue in mammals (Cannon et al. 1982, Lean et al. 1983). The presence of this uncoupling protein (UCP) is the critical means of demonstrating the presence of BAT (Trayhurn 1989), while morphological and histological appearance alone is insufficient evidence for distinguishing between the brown and white functional forms of mammalian fat tissue.

There has been considerable debate as to whether birds possess NST (Connolly et al. 1989) and if they do, whether an avian equivalent to brown adipose tissue is present. Only limited evidence exists for NST in birds (El Halawani et al. 1970, Barré et al. 1986, Sutter and MacArthur 1989) and searches for brown fat using morphological and histological techniques have produced conflicting evidence (Luckenbill and Cohen 1966, Johnston 1971, Freeman 1971, Oliphant 1983, Barré et al. 1986, Olson et al. 1988, Dawson 1989, Saarela et al. 1989, Sutter and MacArthur 1989). Only Saarela et al. (1991) have assayed avian adipose tissue for mitochondrial UCP, finding no evidence for the protein in captive winter-acclimated Ring-necked Pheasants (*Phasianus colchicus*), Japanese Quail (*Coturnix coturnix japonica*), and Rock Doves (*Columba livia*) or in wild House Sparrows (*Passer domesticus*) and Great Tits (*Parus major*) collected in midwinter near Oulu, Finland (65° N). Acclimation to a northern winter should maximize the potential for the development of BAT, and Saarela et al. (1991) argue that the likelihood of birds possessing brown fat is low. However, the authors noted that the most likely avian candidates to possess BAT would be those species that exhibit NST and undergo periods of daily torpor (e.g., hummingbirds and goatsuckers).

The purpose of this study was to assay for the mammalian mitochondrial UCP in Common Poorwills (*Phalaenoptilus nuttallii*: Caprimulgidae), a goatsucker which should use NST, and might be the most likely avian species to possess the fundamental mechanism for NST found in mammals. Poorwills enter deep torpor (body temperature <5.0°C) in both field (Brigham 1992, Kissner and Brigham 1993) and laboratory conditions (reviewed in Csada and Brigham 1992). Further, poorwills are the only bird for which there is evidence for true hibernation (Jaeger 1948). Johnston (1971) found no evidence for BAT in poorwills using histological techniques, but, as noted, the presence of UCP is the definitive test.

METHODS

We analyzed tissues of two wild male poorwills caught on 3 September 1991 near Oliver, British Columbia (bird 1), and 6 September 1992 near Maple Creek, Saskatchewan (bird 2). Poorwills commonly use torpor at both locations at this time of the year (Brigham 1992, Csada and Brigham 1992). The birds were transported to Regina, given only water, and sacrificed within 48 hr.

Adipose tissue was removed from the uropygium, subcutaneous, abdominal, and intrascapular regions of the birds. In addition, the heart of each individual was assayed. All tissues were rapidly frozen and stored at −80°C until being transferred to Aberdeen on dry ice. Unfortunately, the tissue from bird 1 thawed in transit. Because UCP is very stable (Trayhurn, unpubl. data) and because both the thawed and frozen tissues gave the same assay results, we had no reason to discard the data from the thawed bird.

For both birds, the adipose tissues and other organs were homogenized and assayed for cytochrome-c oxidase activity following the procedure of Trayhurn et al. (1987). Mitochondria were prepared for analysis following Trayhurn et al. (1987), with the mitochondrial protein measured using a modified Lowry assay and using bovine serum albumin as a standard.

For the immunological detection of UCP, mitochondrial proteins were solubilized and separated on the basis of molecular weight by SDS-polyacrylamide gel electrophoresis (Trayhurn et al. 1989, Milner and

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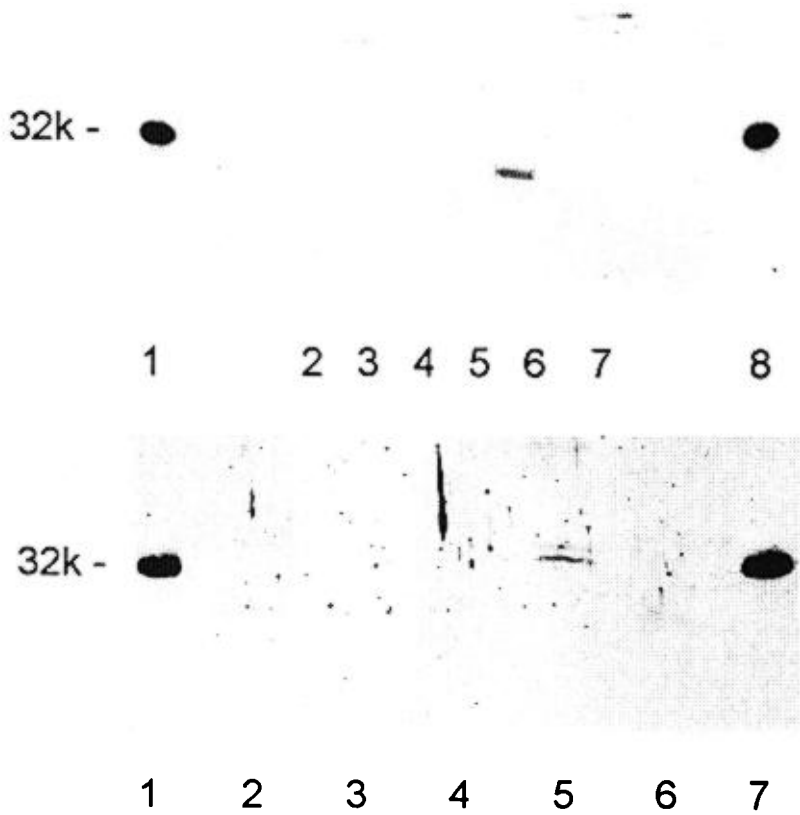

FIGURE 1. Western blot for uncoupling protein in adipose and other tissues removed from two Common Poorwills. (A) bird 1: lanes 1 and 8, uncoupling protein standard (20 ng); lane 2, uropygium; lane 3, pectoral muscle; lane 4, intrascapular fat; lane 5, intestinal fat; lane 6, heart; lane 7, stomach fat. (B) bird 2: lanes 1 and 7, uncoupling protein standard (20 ng); lane 2, skin; lane 3, intrascapular fat; lane 4, intestinal fat; lane 5, heart; lane 6, stomach fat. Five μg of mitochondrial protein was added to each lane.

Trayhurn 1990). We applied 5 μg of mitochondrial protein to each of the wells in a mini vertical slab gel electrophoresis system ("Tall Mighty Small"; Hoefer Scientific Instruments, Newcastle, U.K.). After separation, proteins were transferred to nitrocellulose membranes and probed for UCP by incubating with an anti-(ground squirrel UCP) serum (Milner and Trayhurn 1990) purified from axillary BAT of Richardson's ground squirrel (*Spermophilus richardsoni*) (Milner et al. 1989). The membranes were then incubated with a goat anti-(rabbit IgG) serum conjugated to horseradish peroxidase (Scottish Antibody Production Unit). Antigen-antibody complexes were detected using a highly sensitive enhanced chemiluminescence system ('ECL'; Amersham International, U.K.).

RESULTS

The mitochondrial content of the poorwill adipose tissues was low, as judged both by the yield of the organelle and by tissue cytochrome-c oxidase activity. We found no evidence of immunoreactivity indicative of uncoupling protein in any of the fat depots or organs for either of the birds tested, even following overexposure of the film to the chemiluminescent reaction (Fig. 1).

DISCUSSION

Following the assumption that UCP is the critical diagnostic feature of BAT, we found no evidence for functional "brown" adipose tissue in Common Poorwills. Since the poorwill must be considered a very likely avian candidate to possess NST, we suggest that it is highly unlikely that any avian species has brown adipose tissue, despite some prior evidence to the contrary (e.g., Oliphant 1983, Barré et al. 1986). The possibility that mammalian-like UCP is present in birds but is not immunoreactive with a mammal-derived anti-serum is discussed at length by Saarela et al. (1991) and considered highly unlikely. Although we cannot completely discount the possibility that a mitochondrial UCP exists in birds, but is too dissimilar to the mammalian protein to be detected by mammalian-derived anti-sera, our assay results strongly support the contention that "mammalian" brown adipose tissue is not present in avian species (Saarela et al. 1989, Saarela et al. 1991).

Two important questions remain for birds in general and for poorwills specifically. First, is NST (non-shivering thermogenesis) possible and, if it is, where is the site of thermogenesis? Observations in the field (Brigham, unpubl. data) suggest that torpid birds may rewarm passively using non-shivering thermogenesis although this needs to be evaluated further (e.g., by investigating the beta-adrenergic sensitivity of these birds, Sutter and MacArthur 1989). There is evidence that other species of birds do possess NST and respond thermogenically to exogenous catecholamines (El Halawani et al. 1970, Barré et al. 1986, Sutter and MacArthur 1989).

Assuming that poorwills are capable of NST, then the question of the pathway responsible for thermogenesis must be addressed. For other birds it has been suggested that glucagon may be the mediator (Barré et al. 1989). Whether or not a glucagon-stimulated system would be uncoupled from the normal ATP producing steps of oxidation also needs to be determined.

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MIGRATORY FATTENING IN AN AUSTRALIAN INTRACONTINENTAL MIGRANT[1]

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Key words: Australia; flight range; lipid content; migratory fattening; silvereye; Zosterops lateralis.

Tasmania is a large island separated from the Australian continent by Bass Strait (Fig. 1). Many bird species cross this body of water each year to winter on the Australian mainland before returning to the island to breed in the spring or summer (Ridpath and Moreau 1966). The migration of one such bird, the highly gregarious Grey-breasted Silvereye (*Zosterops lateralis*), is well documented (Ridpath and Moreau 1966, Mees 1974). Migrants include both sexes and both young and old birds, and they may winter as far north as southeastern Queensland (Mees 1974). The crossing of Bass Strait probably presents the most hazardous portion of their migration because refueling stations are limited and westerly winds increase flight costs. Many small birds from the north temperate region carry large lipid stores when preparing for migration (Blem 1980), but this phenomenon of migratory fattening has not been investigated in Australian passerine migrants. I report here the carcass composition of silvereyes captured from a cliff top at Cape Liptrap (38°55′S, 145°55′E) near the southern tip of Victoria (Fig. 1), where the birds were taking off for their return migration over Bass Strait towards Tasmania (Chan and Sutton 1993).

Silvereyes were captured during the austral spring between 29 October and 3 November. A total of 30 birds were caught in mist-nets while taking off for migratory flight. Each freshly-caught bird was immediately weighed to the nearest 0.1 g (=live mass), killed, feathers plucked, dissected, its gut emptied, and weighed again (=fresh mass). The carcass was then frozen for later use.

In the lipid extraction procedure, carcasses were first dried in an oven at 60°C until constant mass was reached, and then homogenized. Mass of dried homogenate represents the dry carcass mass of bird and the water content is the difference between fresh mass and dried homogenate. The homogenate was emptied into a membranous thimble and extracted with 2:1 ethanol : diethyl ether in a Soxhlet apparatus for 4 hr. This is a method consistent with the recommendations by Dobush et al. (1985) for extracting lipid. Mass of the residue is the lean dry content; lipid content is the difference between dry and lean dry mass.

Live and fresh body mass of silvereyes captured from take-off flocks were (mean ± SE) 11.58 ± 0.18 g and 10.79 ± 0.19 g, respectively. The variation in fresh mass and lipid content was independent of sex (F = 0.31, df = 1, 28, $P > 0.05$, ANOVA). Subsequently, data from sex classes were pooled. The average lipid content was 1.79 ± 0.19 g, which represented 15.1% of live mass and 16.2% of fresh mass. An average of 42.6 ± 2.3% of the dry carcass mass was lipid. The relationship between dry carcass mass (4.20 ± 0.15 g) and fresh carcass mass was positive (b = 0.684, r^2 = 0.657, $P < 0.001$). This was due mainly to lipid content in the carcass (b = 1.035, r^2 = 0.958, $P < 0.001$), since the fat-free dry mass (mostly protein, Dobush et al. 1985) did not vary with dry carcass mass (b = −0.034, r^2 = 0.025, $P > 0.1$, Fig. 2). Migratory fattening therefore occurs in the silvereye, since in winter lipid reserve is only half the amount carried by premigratory birds (<8% of fresh mass, Chan, in press). Premigratory lipid reserve of silvereyes is less than that of a long-distance migrant but equivalent to a short-distance migrant (King and Farner 1965, Blem 1980). This is consistent with the overwater distance to be covered, which is approximately 250 km between Cape Liptrap and the northernmost coastline of Tasmania. Water content

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