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Histochemical and biochemical plasticity of muscle fibers in the little brown bat (*Myotis lucifugus*)

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Summary. Fiber composition, and glycolytic and oxidative capacities of the pectoralis, gastrocnemius, and cardiac muscles from active and hibernating little brown bats (*Myotis lucifugus*) was studied. The data were used to test two hypotheses: First, since hibernating bats maintain the capability of flight and make use of leg muscles to maintain a roosting position all winter, the fiber composition of the pectoralis and gastrocnemius muscles should not change with season. Second, we tested the hypothesis of Ianuzzo et al. (in press), who propose that the oxidative potential of mammalian cardiac muscle should increase with increasing heart rate while glycolytic potential should not. Our results indicate that the fiber composition of the pectoralis muscle was uniformly fast-twitch oxidative (FO) regardless of the time of year, as predicted. However, the gastrocnemius muscle exhibited a change in FO composition from 83% in active to 61% in hibernating animals. Contrary to the variable change in histochemical properties with metabolic state, a trend of reduced maximal oxidative (CS) and glycolytic (PFK) potential during hibernation in both flight and leg muscles was apparent. The oxidative potential of flight and leg muscles decreased by 15.2% and 56.5%, respectively, while the glycolytic potential of the same muscles decreased by 23.5% and 60.5%, respectively. As predicted, the glycolytic potential of cardiac muscle remained constant between active and hibernating bats, although there was a significant decrease (22.0%) in oxidative potential during hibernation.

Key words: Histology – Biochemistry – Muscle physiology – Hibernations – Metabolism

Introduction

Bats are reported to have the highest maximal aerobic capacity of all mammals; their maximal oxygen consumption being 2.5–3 times greater than for terrestrial mammals of similar mass (Thomas 1975). During hibernation, the metabolic rate of some insectivorous species falls substantially below the basal or resting level (Henshaw 1970). The large dynamic range of metabolism in some bats is exemplified by the heart rate of *Myotis lucifugus*. During hibernation, the heart rate of this small (8–10 g) insectivorous species ranges from 6 to 32 beats·min^{-1} (Davis and Reite 1967; Henshaw 1970; Nicoll 1964). Heart rates of non-hibernating resting bats are in the range 210–457 beats·min^{-1} (Henshaw 1970; Nicoll 1964; Studier and O'Farrell 1976), while during flight the heart rate of these bats may reach 1368 beats·min^{-1} (Hurst 1966).

Both the contractile system producing muscular force and the metabolic system providing ATP to fuel the contractile system are inherently plastic (Pette 1984; Saltin and Gollnick 1983). One of the major regulatory determinants of these systems in cardiac and skeletal muscle is the physiological demand imposed upon the muscle (Blank et al. 1989; Ianuzzo et al. in press; Saltin and Gollnick 1983). This suggests that the composition of bat flight muscle may change with season, reflecting changes in the dynamic range of metabolism required during and post hibernation. Armstrong et al. (1977), showed that the fiber composition of the pectoralis was homogeneous FO (fast-twitch oxidative) in samples collected in August when bats are active and in March when individuals are preparing to leave hibernacula.

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Abbreviations: FO fast-twitch oxidative; *FG* fast-twitch glycolytic; *SO* slow-twitch oxidative; *Vmax* maximal enzyme activity; *PFK* phosphofructokinase; *CS* citrate synthase

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The purpose of this study was to test two hypotheses. First, since hibernating bats maintain the capability of flight all winter, and the postural function of the leg muscle is not altered with season (Speakman and Racey 1989), the fiber composition of the pectoralis and gastrocnemius muscles should not change. Although Armstrong et al. (1977) showed that the fiber composition of *M. lucifugus* pectoralis did not change between August (active period) and March (end of hibernation), there are no histochemical data for the pectoralis during the middle of the hibernation period when activity should be at a minimum. Further, no comparison of the fiber composition in the gastrocnemius of active and hibernating bats has been made. Second, we tested the hypothesis of Ianuzzo et al. (in press), who propose that the oxidative potential of mammalian cardiac muscle should increase with increasing heart rate while glycolytic potential should not. Ianuzzo and co-workers (Blank et al. 1989; Ianuzzo et al. in press) found that oxidative potential was positively correlated with heart rate for seven species of mammals ranging from mice to cattle, whereas the glycolytic potential was independent of heart rate. Therefore, in *M. lucifugus*, the oxidative potential of heart muscle should decrease in hibernating individuals while the glycolytic potential should remain constant.

Materials and methods

Five hibernating male *M. lucifugus* were collected on 9 November 1986 from an abandoned mine near Renfrew, Ontario (Fenton 1970). Five males constituting the active sample were collected from the Tyandinaga cave near Belleville, Ontario (Thomas et al. 1979) on 15 September 1987 during swarming behaviour prior to hibernation (Fenton 1969). All 10 individuals were at least 1 year of age (based on the degree of ossification of the third-digit metacarpal-phalange joint; Racey 1974). The animals were held in cages for a maximum of 24 h before being sacrificed by cervical dislocation.

The pectoralis and both the medial and lateral heads of the gastrocnemius muscle were excised immediately and from the midbelly of the muscle, a transverse section was cut, mounted on a piece of cork and quick-frozen by immersion in isopentane cooled to the temperature of liquid nitrogen. Serial sections of 10–12 μm were cut in a cryostat at −25 °C and stained for nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR; Novikoff et al. 1961), myofibrillar adenosine triphosphatase (MF-ATPase; Guth and Samaha 1970; Padykula and Herman 1955), and mitochondrial α-glycerophosphate dehydrogenase (αGPDH; Wattenberg and Leong 1960).

Muscle histochemistry was analyzed by classifying a minimum of 300 fibers randomly distributed throughout the muscle sections and comparing distributions with the Chi-square statistic. Muscle fibers were classified as FO, fast-twitch glycolytic (FG), or slow-twitch oxidative (SO) using a modification of the system proposed by Peter et al. (1972). FO and FG fibers stained darkly for MF-ATPase when preincubated at pH 10.3 and lightly when preincubated at pH 4.4. SO fibers stained in the opposite way to fast fibers for the two preincubation media. FO fibers stained dark for NADH-TR while FG fibers stained light.

The remaining parts of the excised flight and leg muscles and the heart were used to spectrophotometrically determine the maximal enzyme activity of phosphofructokinase (PFK) and citrate synthase (CS) using the methods of Shonk and Boxer (1964) and Srere (1969), respectively. These enzymes are representative of the relative glycolytic and oxidative potentials of these muscles (Ianuzzo et al. in press). The PFK assay was run in a phosphate buffer to decrease the changes in pH over time, associated with the bicarbonate system used by Shonk and Boxer (1964). To ensure that the maximal activity of PFK in the homogenate was measured, the substrate concentration was doubled while reducing the homogenate (enzyme) by one-half to check if the rate of reaction remained the same. This does not indicate whether or not PFK is phosphorylated differently in the bat muscles during their active or hibernating period but it does indicate maximal activity is being determined. Mean maximal activities (μmoles·g muscle wet weight^{-1}·min^{-1}) were compared using a one-way ANOVA. Protein determinations indicated no difference in water content between the muscles of hibernating and active bats.

To serve as standards for comparison, samples of male Sprague-Dawley rat plantaris muscles were obtained in September and November and simultaneously assayed using the same histochemical and biochemical analyses performed on the bat tissues.

Results

The rat plantaris muscle fiber composition was very similar to that found by Ianuzzo et al. (1976) and Armstrong et al. (1977) and did not change between samples in this study ($\chi^2 = 3.5$, $p > 0.05$; Table 1). This indicates that the staining procedures were consistent between studies and samples.

The fiber composition of the pectoralis muscle from both hibernating and active *M. lucifugus* was uniformly FO (Table 1). Visually, the fibers were no different than those illustrated in Figs. 1 and 3 of Armstrong et al. (1977). The gastrocnemii, however, did not consist of a uniform fiber population, and there were differences in composition between active and hibernating bats (Table 1). Gastrocnemii from active bats were composed of 85% FO fibers, significantly different ($\chi^2 = 171.8$, $p < 0.001$) from the 62% FO fiber composition in hibernating individuals. In both samples, the remaining fibers were SO.

The glycolytic staining of *M. lucifugus* pectoralis muscle showed no difference between the fibers of active

Table 1. Mean (±SD) fiber composition (%) of bat pectoralis and gastrocnemius muscles for active and hibernating individuals. Rat plantaris muscle fiber composition was determined during both characterizations of bat tissue. *n* represents number of fibers counted/number of individual animals

	M. lucifugus		*R. norvegicus* Plantaris
	Pectoralis	Gastrocnemius	
Hibernating			
FO	100±0	61.4±7.4	39
FG	0±0	0±0	50
SO	0±0	38.6±7.4	11
n	1589/5	1228/4	308/1
Active			
FO	100±0	82.8±9.0	47
FG	0±0	0±0	43
SO	0±0	17.2±9.0	10
n	1586/5	1528/5	304/1

Table 2. Mean (±SD) activity for PFK (phosphofructokinase) and CS (citrate synthase) enzyme assays of bat and rat muscles. Units for both enzymes are $\mu mol \cdot g^{-1} \cdot min^{-1}$. F value represents values for one way analysis of variance between means

	M. lucifugus						*R. norvegicus*			
	Heart		Pectoralis		Gastrocnemius		Heart		Plantaris	
	PFK	CS	PFK	CS	PFK	CS	PFK	CS	PFK	CS
Active										
mean	52	246	68	217	38	85	34.9	111.5	57.8	25.4
SD	8.6	28.7	11.1	33.8	6.1	13.5	–	–	–	–
n	5	5	5	5	5	5	1	1	1	1
F value	0.2	7.9 *	8.6 *	3.5	65.1 *	29.2 *				
Hibernating										
mean	50	192	52	184	15	37	34.5	164.9	86.4	13.1
SD	8.8	32.4	5.6	19.3	1.5	14.5	–	–	–	–
n	5	5	5	5	5	5	1	1	1	1

* represents significant differences ($p < 0.05$) between active and hibernating samples

and hibernating individuals (100% of the fibers stained dark for αGPDH in both samples). The glycolytic stain of *M. lucifugus* gastrocnemius muscle differed between active and hibernating bats and differed from the pectoralis. Dark-staining fibers comprised 49% in the active sample and 67% in the hibernating sample (n = 650 and 900 fibers, respectively). The ratio of dark- to light-staining gastrocnemius fibers was significantly different between summer and winter samples ($\chi^2 = 54.9$, $p < 0.001$).

Maximal PFK activity of the pectoralis and gastrocnemius muscles, but not cardiac muscle, was significantly less in hibernating individuals (Table 2). Maximal CS activity was significantly lower in the cardiac and gastrocnemius muscles of hibernating versus active individuals, but not in the pectoralis muscle.

Discussion

Our results show that the uniform FO fiber composition of *M. lucifugus* pectoralis muscle does not change with season, supporting the data of Armstrong et al. (1977). Foehring and Hermanson (1984) reported that the pectoralis muscle of another small insectivorous bat, *Tadarida brasiliensis*, was uniformly FO and suggested that this reflects the high metabolic demands of flight over extended periods of the night (> 300 min). If Foehring and Hermanson's hypothesis is correct, then the flight muscle histochemistry of *M. lucifugus* leads to the prediction that individuals should forage for long periods each night. However, the foraging times of individual *M. lucifugus* are considerably less than 300 min per night (Barclay 1982). To date, no histochemical work has been done for species with short flight times (< 120 min) such as *Eptesicus fuscus* (Brigham and Fenton 1986) or *Scotophilus borbonicus* (Barclay 1985; Fenton et al. 1985; Fenton and Rautenbach 1986).

The fiber-type composition of *M. lucifugus* gastrocnemius muscle did change between hibernating and active individuals, contrary to our predictions. Hibernating individuals had a significantly greater proportion of slow-twitch fibers than individuals in the active sample. However, comparable to the results of Foehring and Hermanson (1984), we found no evidence for FG fibers in any of the muscles we studied. Since hibernating bats remain still for long periods of time, the need for fast-twitch fibers in the leg may be lessened. Further, it is well documented that low-level chronic stimulation (such as hanging by the feet for long periods of time) causes a shift from fast to slow muscle fiber composition (Pette and Vrbova 1985). A complete conversion to SO fibers does not occur for the same reasons mentioned for flight muscles; that is, the maintenance of flight capabilities through the winter. The occasional midwinter activity of *M. lucifugus* may require fast-twitch capabilities in the gastrocnemius muscle.

Unlike Armstrong et al. (1977), we found a general trend for reduced maximal potential of both oxidative and glycolytic capacity during hibernation using PFK and CS activities as assays. Whether this difference is due to the slightly different methods used or results from sampling the bats at different stages in the hibernation period is unknown. For all three bat muscles studied, and both enzymes, hibernating individuals had a lower maximal activity, with four of the six comparisons being statistically significant (Table 2).

The interpretaton of our results merits care since qualitative alteration in isozyme pattern and changes in specific activity and thermal sensitivity occur in flight muscle pyruvate kinase (PK) of *M. lucifugus* during hibernation (Borgmann and Moon 1976; see also Brabec and McColloch 1970 for a discussion of PFK). Borgmann and Moon (1976) and Moon (1978) showed that the activity levels of PK increased while lactate dehydrogenase decreased in hibernating bats, although the magnitude of change was dependent on both temperature and substrate concentration. Our results show that although the fiber composition of *M. lucifugus* pectoralis

muscle does not change, the glycolytic potential of the muscle decreases. The change in gastrocnemius fiber composition is accompanied by a significant reduction in both glycolytic and oxidative capacity.

The stability in glycolytic potential of cardiac muscle from active and hibernating bats, and the significant decrease in oxidative potential of cardiac muscle in hibernating individuals fits our predictions and the data of Ianuzzo et al. (in press). These results provide support for the hypothesis that oxidative, but not glycolytic, capacity of the heart is regulated by cardiac activity.

In conclusion, the fiber composition of *M. lucifugus* pectoralis is homogeneous FO throughout the hibernation period, but there are fiber composition changes in the gastrocnemius during the same period. These histochemical changes occur while there is a general trend for reduced maximal oxidative and glycolytic potential during the early part of hibernation.

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References

Armstrong RB, Ianuzzo CD, Kunz TH (1977) Histochemical and biochemical properties of flight muscle fibers in the little brown bat, *Myotis lucifugus*. J Comp Physiol 119:141–154

Barclay RMR (1982) Night roosting behavior of the little brown bat, *Myotis lucifugus*. J Mamm 63:464–474

Barclay RMR (1985) Foraging behaviour of the African insectivorous bat, *Scotophilus leucogaster*. Biotropica 17:65–70

Blank S, Chen V, Hamilton N, Salerno TA, Ianuzzo CD (1989) Biochemical characteristics of mammalian Myocardia. J Mol Cell Cardiol 21:367–373

Borgmann AI, Moon TW (1976) Enzymes of the normothermic and hibernating bat, *Myotis lucifugus*: Temperature as a modulator of pyruvate kinase. J Comp Physiol 107:185–199

Brabec MJ, McColloch RJ (1970) Phosphofructokinase activity in the brown fat, liver, and heart of hibernating and homeothermic thirteen-lined ground squirrels (*Citellus tridecemlineatus*). Int J Biochem 1:557–560

Brigham RM, Fenton MB (1986) The influence of roost closure on the roosting and foraging behaviour of *Eptesicus fuscus* (Chiroptera: Vespertilionidae). Can J Zool 64:1128–1133

Davis WH, Reite OB (1967) Responses of bats from temperate regions to changes in ambient temperature. Biol Bull 132:320–328

Fenton MB (1969) Summer activity of *Myotis lucifugus* (Chiroptera: Vespertilionidae) at hibernacula in Ontario and Quebec. Can J Zool 47:597–602

Fenton MB (1970) Population studies of *Myotis lucifugus* (Chiroptera: Vespertilionidae) in Ontario. ROM Life Sci Contr 77 34pp

Fenton MB, Brigham RM, Mills AM, Rautenbach, IL (1985) The roosting and foraging areas of *Epomophorous wahlbergi* (Pteropodidae) and *Scotophilus viridis* (Vespertilionidae) in Krüger National Park, South Africa. J Mamm 66:461–468

Fenton MB, Rautenbach IL (1986) A comparison of the roosting and foraging behaviour of three species of African insectivorous bats (Rhinolophidae, Vespertilionidae, Molossidae). Can J Zool 64:2860–2867

Foehring RC, Hermanson JW (1984) Morphology and histochemistry of flight muscles in free-tailed bats, *Tadarida brasiliensis*. J Mamm 65:388–394

Guth L, Samaha FJ (1970) Procedure for the histochemical demonstration of actomyosin ATPase. Exp Neurol 28:365–367

Henshaw RE 1970. Thermoregulation in bats. In: Slaughter BH, Walton DW (eds) About bats. Southern Methodist University Press, Dallas, pp 188–232

Hurst RN (1966) Seasonal physiological responses of the bat *Myotis lucifugus*. Unpublished PhD Thesis, Purdue University, Lafayette Indiana

Ianuzzo CD, Gollnick PD, Armstrong RB (1976) Histochemical adaptations of rat skeletal muscle fibers to a long-term power overload condition. Life Sci 19:1517–1524

Ianuzzo CD, Blank S, Hamilton N, O'Brien P, Chen V, Brotherton S, Salerno TA (in press) The relationship of myocardial chronotropism to the biochemical capacities of mammalian hearts. Biochemistry of Exercise VII. Human Kinetics Publishers, Champaign Illinois

Moon TW (1978) Enzymes of heterotherms: LDH of hibernating and normothermic little brown bats, *Myotis lucifugus*. Comp Biochem Physiol 59B:183–190

Nicoll PA (1964) Arterial pressures in the bat *Myotis lucifugus*. Fed Proc Fed Am Soc Exp Biol 23:253

Novikoff AB, Shin W, Drucker J (1961) Mitochondrial localization of oxidative enzymes: staining results with two tetrazolium salts. J Biophys Biochem Cytol 9:47–61

Padykula HA, Herman E (1955) The specificity of the histochemical methods of adenosine triphosphatase. J Histochem Cytochem 3:170–195

Peter JB, Barnard RJ, Edgerton VR, Gillespie CA, Stempel KE (1972) Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. Biochem 11:2627–2634

Pette D (1984) Activity-induced fast to slow transitions in mammalian muscle. Med Sci in Sports Exer 16:517–528

Pette D, Vrbova G (1985) Neural control of phenotypic expression in mammalian muscle fibers. Muscle and Nerve 8:676–689

Racey PA (1974) Ageing and assessment of reproductive status of Pipistrelle bats, *Pipistrellus pipistrellus*. J Zool Lond 173:264–271

Saltin B, Gollnick PD (1983) Skeletal muscle adaptability: Significance for metabolism and performance. In: Peachy LD, Adrian RH, Geiger SR (eds) Handbook of physiology. Sec 10 Skeletal muscle Bethesda: Amer Physiol Sci pp 555–632

Shonk CE, Boxer GE (1964) Enzyme patterns in human tissue. I. method for determination of glycolytic enzymes. Cancer Res 24:709–721

Speakman JR, Racey PA (1989) Hibernal ecology of the Pipistrelle bat: Energy expenditure, water requirements and mass loss, implications for survival and the function of winter emergence flights. J Anim Ecol 58:797–813

Srere PA (1969) Citrate synthase. Meth Enzymol 13:3–8

Studier EH, O'Farrell JJ (1976) Biology of *Myotis thysanodes* and *M. lucifugus* (Chiroptera: Vespertilionidae). III. Metabolism, heart rate, breathing rate, evoporative water loss and general energetics. Comp Biochem Physiol 54:423–432

Thomas SP (1975) Metabolism during flight in two species of bats, *Phyllostomus hastatus* and *Pteropus gouldii*. J Exp Biol 63:273–293

Thomas DW, Fenton MB, Barclay RMR (1979) Social behaviour of the little brown bat, *Myotis lucifugus*. I. Mating behaviour. Behav Ecol Sociobiol 6:129–136

Wattenberg LW, Leong JL (1960) Effects of coenzyme Q_{10} and menadione on succinate dehydrogenase activity as measured by tetrazolium salt reduction. J Histochem Cytochem 8:296–303