

Maintenance of the pectoralis muscle during hibernation in the big brown bat, *Eptesicus fuscus*

Marshall E. Yacoe*

Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109, USA

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Summary. The relationship between pectoralis muscle mass and body mass is examined throughout the annual body mass cycle in *Eptesicus fuscus* in order to evaluate muscle maintenance during hibernation. *E. fuscus* undergoes large fluctuations in body mass during the year due to pregnancy, parturition, prehibernation fattening, and hibernation (Table 1). Parallel changes occur in pectoralis muscle mass and total pectoralis protein mass (Table 2). The strong correlation between log pectoralis mass and log body mass (Fig. 3) and the lack of correlation between pectoralis mass and forearm length (Fig. 1, 2) suggest that the seasonal variation in pectoralis muscle mass represents a compensatory response to changing body mass. In active bats this relationship closely resembles the compensatory response predicted by flight theory.

Both pectoralis muscle mass and body mass decrease significantly during four months of hibernation (Tables 1, 2). Although pectoralis mass and body mass are not significantly correlated after four months of hibernation the values fall within the range of observations in active summer bats (Fig. 3), indicating an approximate maintenance of the pectoralis mass/body mass relationship. The lack of correlation in hibernating bats may result from differences in the function of pectoralis muscle during activity (powering flight) and hibernation (thermogenesis and supply of gluconeogenic precursors). The significant elevation of pectoralis muscle protein concentration during prehibernation fattening and its progressive decrease during hibernation (Fig. 4) support the idea that the pectoralis muscle serves as an important source of gluconeogenic precursors during hibernation.

Introduction

During summer the big brown bat, *Eptesicus fuscus*, is a nocturnal, aerial insectivore. Foraging flights require a sustained, high level of power output, virtually all of which is provided by the two large pectoralis muscles. Pectoralis muscle mass and body mass are strongly correlated across a wide size range in bats (Greenewalt 1962), implying that a certain amount of muscle is needed to support flight in a bat of a given body mass. This relationship is predicted in flight theory (Pennycuik 1975) and supported by numerous observations in birds (Greenewalt 1962; Tucker 1973; Greenewalt 1975; Marsh and Storer 1981). It follows that in order to retain the ability to fly, the relationship between pectoralis mass and body mass must also be maintained throughout hibernation.

Eptesicus fuscus hibernates for up to five months in southeastern Michigan due to the absence of its primary food, flying insects. The energy requirements of hibernation are supplied mainly by fat (Beer and Richards 1956; Dodgen and Blood 1956). However, mammals cannot live on fat alone. The oxidation of fats in the citric acid cycle depends on a constant input of 3- and 4-carbon intermediates (Spydevold et al. 1976; Lee and Davis 1979), which can only be derived from glucose, amino acids, or glycerol (Lehninger 1975). In addition, the central nervous system requires glucose even during prolonged fasting (Owen et al. 1967). Hibernators which do not cache food must provide these requirements from endogenous sources. Since glycogen stores are small and do not undergo net depletion during hibernation in the mammals which have been studied (Dodgen and Blood 1956; Leonard and Wimsatt 1959; Troyer 1959; Galster and Morrison 1970, 1975;

* Present address: Marine Biology Division A-002, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093, USA

Yacoe 1982; Zimmerman 1982), glucose and citric acid cycle intermediates must be synthesized from amino acids and/or glycerol. However, the availability of glycerol is limited by the rate of fat oxidation. It is inherent in the chemical composition of triglycerides that glycerol can provide only a small contribution to overall metabolism. Demand in excess of the amount which can be supplied by glycerol must be met by amino acids derived from tissue proteins. Skeletal muscle is by far the largest protein reserve and the most important source of gluconeogenic precursors during prolonged fasting in normothermic mammals (Snell 1980). However, the catabolism of muscle protein results in atrophy and loss of function. The present study examines the extent to which the degradation of skeletal muscle protein may meet metabolic needs during hibernation in *E. fuscus*. It was hypothesized that successful hibernation might depend on the balance between the use of protein for metabolic needs and the maintenance of sufficient muscle function to support normal activity upon arousal in the spring.

The extent of muscle protein catabolism during hibernation is evaluated here in light of the general decrease in body mass during hibernation (Beer and Richards 1956). The relationship between pectoralis muscle mass and body mass is examined throughout the annual body mass cycle in *E. fuscus* and is compared with that predicted by flight theory (Pennycuik 1975). These comparisons are used to determine the extent to which short term changes in body mass (as in pregnancy and prehibernation fattening) are compensated by changing muscle mass. Deviations of the observed relationship from the predicted one should indicate the extent to which muscle mass may vary from the predicted value and still be capable of supporting flight. The relationship between pectoralis muscle mass and body mass in actively flying bats is then used as an index for evaluating the maintenance of muscle during hibernation. Finally, muscle protein content is measured to provide an indication of the extent of protein catabolism during hibernation.

Materials and methods

Animals

All of the bats used in this study were collected in attics and barns in the vicinity of Ann Arbor, Michigan. 'Summer' animals were collected from early June through the middle of August. Bats used for the prehibernation and hibernation measurements were collected in late September and early October. Animals used for prehibernation measurements were sampled

immediately. The remaining bats were housed in cages in a darkened chamber at 5–7 °C and 100% relative humidity. These conditions approximate those reported for natural hibernacula (Beer and Richards 1956) and bats held in this manner follow a similar course of mass loss to that of free living animals in hibernation (Beer and Richards 1956). The bats used here had been hibernating for at least three months prior to sampling.

Physical measurements

Bats were killed by a blow to the head. Body mass was measured to 0.01 g using a Mettler top loading balance. The left pectoralis muscle was rapidly excised, weighed to 0.01 g and used for protein determinations. The right pectoralis muscle was carefully dissected and weighed to 0.001 g using a Mettler analytical balance. The pectoralis mass reported here is twice the measured mass of the right pectoralis. Forearm length was recorded to 0.5 mm.

Body composition

Fat mass was determined by the method of Carey et al. (1978). Carcasses were dried to constant mass using a Virtis lyophilizer. The dry carcasses were weighed to the nearest 0.01 g, shredded with scissors, and packed into cellulose extraction thimbles. Carcass neutral lipids (predominantly fats) were extracted with petroleum ether using a Soxhlet apparatus. The extracted material was then oven-dried for a week at 90 °C and weighed to 0.01 g. Fat mass was taken to be the difference in carcass mass with fat extraction. Fetal mass was determined directly by dissection of both fetuses and the placenta. The combined mass of this material was measured to 0.01 g. Lean mass was calculated by subtracting fat mass (and fetus mass) from total body mass.

Protein determinations

Pectoralis muscle protein content was estimated using the biuret procedure described by Watters (1978). Muscle samples were homogenized in 10 ml/g tissue of 100 mM potassium phosphate, 2 mM EDTA, pH 7.3 for 10 s at a setting of 50 on a Tekmar Tissuemizer. Duplicate diluted portions of this homogenate were used for the protein determination and each value reported is the mean of two independent measurements. Values for the total mass of pectoralis muscle protein represent the product of protein concentration and tissue mass.

Statistics

Multiple comparisons of mean values were made by analysis of variance, except where sample variances differed significantly. In those cases, multiple comparisons were made by the Kruskal-Wallis test and pairwise comparisons were made by the Wilcoxon rank sum test. The relationships of pectoralis mass with body mass and total pectoralis protein with body mass were evaluated using analysis of covariance. Null hypotheses were rejected at the 0.05 level.

Results

Eptesicus fuscus undergoes continuous, large fluctuations in body mass during the year (Table 1). However, for purposes of simplicity the yearly body mass cycle is broken into four periods: preg-

Table 1. Physical characteristics of *Eptesicus fuscus* during the annual body mass cycle

Parameter	Pregnant	Nonpregnant	Prehibernation	Hibernation
Total body mass (g)	22.35 ± 0.32 ^a (n = 17)	12.86 ± 0.37 ^b (n = 28)	19.12 ± 1.05 ^a (n = 11)	14.10 ± 0.46 ^b (n = 14)
Fat mass (g)	0.88 ± 0.08 ^a (n = 17)	0.40 ± 0.03 ^b (n = 18)	4.28 ± 0.59 ^a (n = 11)	1.45 ± 0.27 ^b (n = 14)
Fetal mass (g)	5.26 ± 0.35 (n = 17)	–	–	–
Lean mass (g)	16.13 ± 0.25 ^a (n = 17)	12.02 ± 0.42 ^b (n = 18)	14.84 ± 0.51 ^a (n = 11)	12.65 ± 0.29 ^b (n = 14)
Lean dry mass (g)	4.82 ± 0.16 ^a (n = 17)	3.87 ± 0.20 ^b (n = 18)	4.66 ± 0.20 ^a (n = 11)	3.89 ± 0.15 ^b (n = 14)
% Water ^c	69.8 ± 0.7 (n = 17)	69.1 ± 0.2 (n = 18)	68.7 ± 0.3 (n = 11)	69.3 ± 0.7 (n = 14)
Forearm length (mm)	46.7 ± 0.3 (n = 17)	45.9 ± 0.4 (n = 18)	45.4 ± 0.4 (n = 11)	45.4 ± 0.5 (n = 14)

All values are presented as mean ± SEM, 'n' denotes sample size

^a Significantly different from the summer nonpregnant value ($P < 0.01$)

^b Significantly different from the prehibernation value ($P < 0.01$)

^c The percent of lean mass accounted for by water

nant, summer nonpregnant, prehibernation fattening, and hibernation. Mean body mass varies significantly among these four groups ($P < 0.001$). Pregnant females were captured in early June when they were very near term in order to see the maximum effect of pregnancy on total body mass and related parameters. The mean body mass of pregnant females (22.35 ± 0.32 g) is significantly greater ($P < 0.001$) than that of nonpregnant summer animals (12.86 ± 0.37 g). The summer nonpregnant group is rather heterogeneous, including post-partum females, males, and some first year animals captured in late August. The body mass of adult males, post-partum females, and first year bats do not differ significantly from one another. Animals in the prehibernation group were captured in late September and early October when they were quite fat but still active. The mean body mass (19.12 ± 1.05 g) in prehibernation bats is significantly greater ($P < 0.001$) than in nonpregnant summer animals. Hibernating bats were sacrificed after 3–4 months in hibernation. Body mass in this group (14.10 ± 0.46 g) is significantly less ($P < 0.001$) than in prehibernation animals.

The seasonal cycle in total body mass is largely accounted for by changes in fat mass and fetal mass (Table 1). Fat mass reaches a peak during prehibernation fattening (4.28 ± 0.59 g) and then gradually decreases during hibernation. Fetal mass a few days prior to parturition (5.26 ± 0.35 g) also represents a large fraction of body mass. However, the changes in body mass shown in Table 1 also

include significant seasonal changes in lean mass ($P < 0.001$).

Both pectoralis muscle mass and total pectoralis protein mass are significantly greater during pregnancy ($P < 0.001$) and prehibernation fattening ($P < 0.001$) than in nonpregnant summer bats (Table 2). After four months of hibernation both pectoralis mass ($P < 0.001$) and total pectoralis protein mass ($P < 0.001$) are significantly reduced relative to prehibernation bats (Table 2). The differences in muscle mass and protein mass between groups are not due to sampling different sized bats, since mean forearm length does not differ significantly between groups (Table 1). There is no overall correlation between pectoralis mass and forearm length (Fig. 1). However, within groups differences in forearm length seem to account for part of the variation in muscle mass. Pectoralis muscle mass is significantly correlated with forearm length in pregnant ($r = 0.60$, $P < 0.01$) and hibernating ($r = 0.64$, $P < 0.01$) bats, but not in nonpregnant or prehibernation animals. The extent of seasonal differences in muscle mass and protein mass (between prehibernation and hibernation bats) are compared with the effect of body size on these parameters in Fig. 2. Pectoralis muscle mass and total pectoralis protein mass are significantly correlated with forearm length in hibernating bats, but the seasonal changes in these parameters far outweigh the differences related to variation in body size.

Pectoralis muscle mass closely follows body

Table 2. Characteristics of the pectoralis muscle during the annual body mass cycle

Parameter	Pregnant	Nonpregnant	Prehibernation	Hibernation
Pectoralis mass (g) ^c	1.26 ± 0.03 ^a (n=18)	0.87 ± 0.04 ^b (n=18)	1.20 ± 0.06 ^a (n=11)	0.88 ± 0.03 ^b (n=14)
Total pectoralis protein (g)	0.31 ± 0.01 ^a (n=18)	0.21 ± 0.01 ^b (n=18)	0.41 ± 0.03 ^a (n=11)	0.22 ± 0.01 ^b (n=14)
Protein content (mg/g)	248.1 ± 4.4 (n=18)	249.7 ± 6.6 ^b (n=18)	336.1 ± 10.9 ^a (n=11)	302.2 ± 5.7 ^{ab} (n=14)
% of body mass ^d	5.77 ± 0.12 ^{ab} (n=18)	6.60 ± 0.12 (n=28)	6.29 ± 0.14 (n=11)	6.27 ± 0.23 (n=14)

All values are presented as mean ± SEM, 'n' denotes sample size

^a Significantly different from the summer nonpregnant value ($P < 0.01$)

^b Significantly different from the prehibernation value ($P < 0.01$)

^c Twice the mass of the right pectoralis muscle (see Materials and Methods)

^d (Pectoralis mass/total body mass) × 100. Body mass values are as reported in Table 1

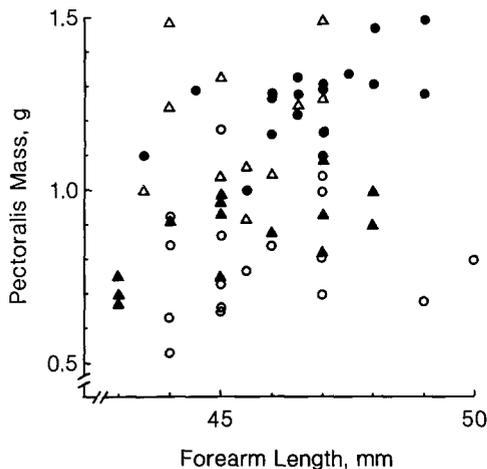


Fig. 1. The mass of the pectoralis muscle as a function of forearm length. The *open circles* are nonpregnant summer bats, the *closed circles* are pregnant bats, the *open triangles* are bats during prehibernation fattening, and the *closed triangles* are bats after four months of hibernation

mass throughout the year. Despite a large variation in total body mass, the proportion of body mass represented by pectoralis muscle mass does not differ significantly between the nonpregnant summer, prehibernation, and hibernation groups (Table 2). However, this proportion is significantly lower ($P < 0.001$) in pregnant females. The relationship between pectoralis muscle mass and body mass is shown in more detail in Fig. 3. There is a strong overall correlation between pectoralis mass and body mass ($r = 0.91$), but this relationship differs between groups of animals. The simple linear regression of log pectoralis mass (m_p) on log body mass (m_b) in nonpregnant summer bats

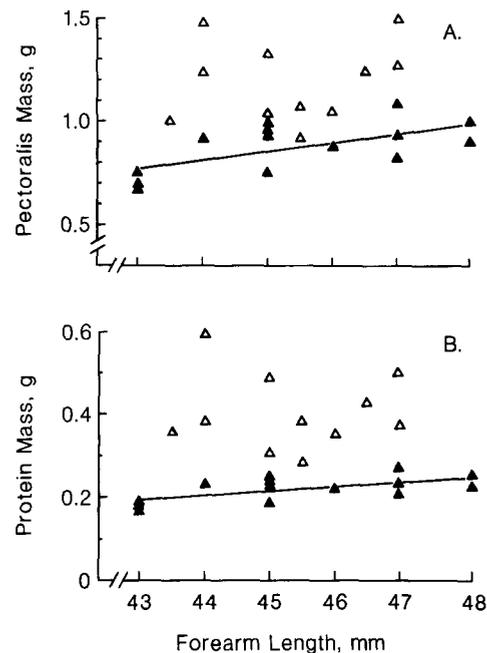


Fig. 2. Factors influencing total pectoralis muscle mass and total pectoralis protein mass. *Upper panel:* The relationship between pectoralis muscle mass and forearm length in prehibernation (*open triangles*) and hibernating (*closed triangles*) bats. The line represents the simple linear regression of pectoralis mass on forearm length in hibernating bats. The regression equation is: $m_p = 0.04 L - 1.13$ ($r = 0.64$, $P < 0.01$), where m_p = pectoralis muscle mass in g and L = forearm length in mm. There is no significant correlation between these parameters in prehibernation bats. *Lower panel:* The relationship between total pectoralis protein mass and forearm length in prehibernation (*open triangles*) and hibernating (*closed triangles*) bats. The line represents the simple linear regression of protein mass on forearm length in hibernating bats. It is described by the equation, $m_{prot} = 0.01 L - 0.28$ ($r = 0.63$, $P < 0.01$), where m_{prot} = total pectoralis protein mass in g and L = forearm length in mm. There is no significant correlation in prehibernation bats

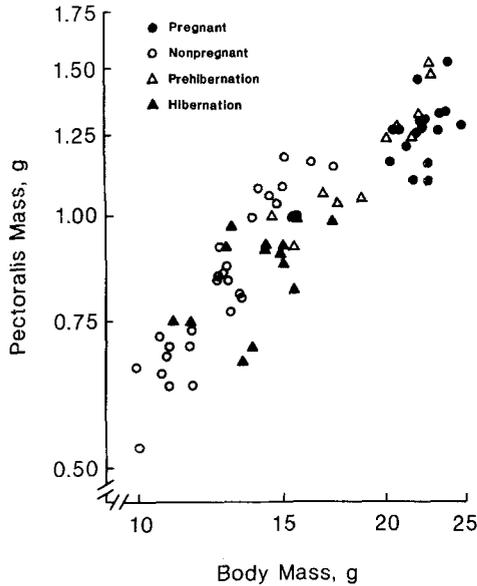


Fig. 3. The relationship between pectoralis muscle mass and body mass through the yearly body mass cycle in *Eptesicus fuscus*. Both scales are logarithmic

is described by the equation:

$$\log m_p = 1.37 \log m_b - 1.59 \quad (1)$$

($r=0.93$, $P<0.001$). As body mass increases during pregnancy and prehibernation fattening, pectoralis mass does not increase at the rate predicted by Eq. 1. The slope of the regression of log pectoralis mass on log body mass in pregnant animals:

$$\log m_p = 0.62 \log m_b - 0.74 \quad (2)$$

($r=0.61$, $P<0.01$) is significantly lower ($P<0.001$) than in nonpregnant summer bats. Similarly, the slope of the regression of log pectoralis mass on log body mass in prehibernation bats:

$$\log m_p = 0.82 \log m_b - 0.98 \quad (3)$$

($r=0.93$, $P<0.001$) is significantly lower ($P<0.005$) than in nonpregnant summer bats. Pectoralis muscle mass is not significantly correlated with body mass in hibernating bats ($r=0.50$, $P=0.070$). However, the distribution of values is very similar to that for nonpregnant summer bats (Fig. 3). Additionally, neither mean pectoralis mass (Table 1), mean body mass (Table 1), nor the ratio of pectoralis mass to body mass (Table 2) differ significantly between nonpregnant summer bats and hibernating bats.

Pectoralis muscle protein concentration in prehibernation bats ($336.1 \pm 10.9 \text{ mg} \cdot \text{g muscle}^{-1}$) is significantly greater than in pregnant ($248.1 \pm 4.4 \text{ mg} \cdot \text{g muscle}^{-1}$; $P<0.001$) and non-

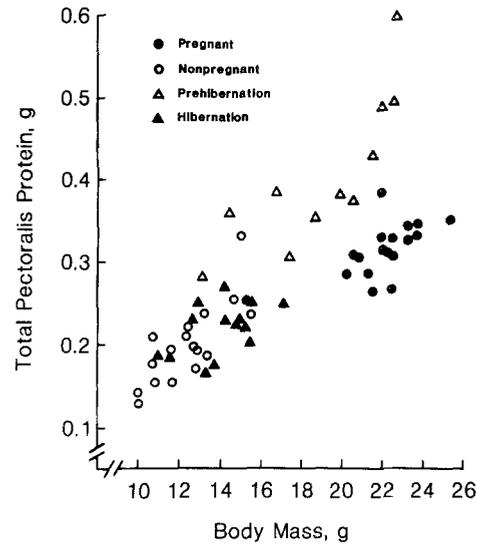


Fig. 4. The relationship between total pectoralis muscle protein mass and body mass through the yearly body mass cycle in *Eptesicus fuscus*

pregnant summer bats ($249.7 \pm 6.6 \text{ mg} \cdot \text{g muscle}^{-1}$; $P<0.001$). After four months of hibernation, pectoralis muscle protein concentration ($302.2 \pm 5.7 \text{ mg} \cdot \text{g muscle}^{-1}$) is significantly less than in prehibernation bats ($P<0.01$), but it is still greater than in summer bats ($P<0.01$). The relationship between total pectoralis protein mass and total body mass is shown in Fig. 4. Not surprisingly, this is very similar to the relationship between pectoralis muscle mass and total body mass (Fig. 3). However, as a result of the increased pectoralis muscle protein concentration, the slope of the regression of total pectoralis protein mass (m_{prot}) on body mass (m_b) in prehibernation bats:

$$m_{\text{prot}} = 0.023 m_b - 0.024 \quad (4)$$

($r=0.80$, $P<0.005$) is identical with that for nonpregnant summer bats:

$$m_{\text{prot}} = 0.023 m_b - 0.083 \quad (5)$$

($r=0.79$, $P<0.001$). Total pectoralis protein and body mass are not significantly correlated in hibernating bats ($r=0.50$, $P=0.066$). However the distribution of values falls within the range of observations for nonpregnant summer bats (Fig. 4). The regression of total pectoralis protein mass on body mass in pregnant bats:

$$m_{\text{prot}} = 0.010 m_b - 0.085 \quad (6)$$

($r=0.64$, $P<0.005$) differs significantly with Eqs. (4) and (5).

Discussion

This paper addresses the hypothesis that successful hibernation in *Eptesicus fuscus* depends partly on the balance between the use of pectoralis muscle protein for metabolic needs and the maintenance of sufficient muscle to support flight upon arousal in the spring. However, the relevance of this hypothesis is dependent on the extent to which muscle protein is catabolized during hibernation. Both pectoralis muscle mass and total pectoralis protein mass are significantly lower in bats sampled after four months of hibernation than in prehibernation bats (Table 2). These data indicate that muscle mass is decreased during hibernation due to protein catabolism. The prehibernation and hibernating groups do not differ in body size (forearm length = 45.4 ± 0.4 ; 45.4 ± 0.5 mm, respectively). Moreover, there is no overall relationship between pectoralis mass and forearm length (Fig. 1), indicating that the extent of seasonal changes in pectoralis mass is much larger than the variation due to differences in body size. The nature of the changes in pectoralis mass and protein mass during hibernation are further illustrated in Fig. 2. Bats in all size classes lose muscle mass and protein during hibernation.

Protein metabolism during hibernation has received very little attention. The data presented here are the first to show that muscle mass and protein decrease during hibernation. However, observations that hibernating black bears have decreased but significant rates of urine formation and nitrogen excretion (Nelson et al. 1973) and that arctic ground squirrels undergo a significant loss of lean body mass during hibernation (Galster and Morrison 1976) are consistent with the idea that protein is catabolized during hibernation. This is further strengthened by studies of humans which show significant, although greatly reduced, rates of protein degradation and nitrogen excretion during prolonged fasting (Cahill 1976; Felig 1979; Millward 1979).

Protein catabolism during hibernation may serve two major functions. First, it could provide precursors for gluconeogenesis. Glucose is a required nutrient in normothermic mammals (Cahill 1976; Felig 1979; Owen et al. 1967), although its necessity during hibernation is uncertain (Zimmerman 1982). Since glucose is only stored in small quantities as liver and muscle glycogen in *E. fuscus* (Yacoe 1982) and these stores are not depleted during hibernation, glucose use should depend on gluconeogenesis. Second, protein catabolism could provide a source of citric acid cycle intermediates.

Mitochondria are unable to oxidize fatty acids in the absence of citric acid cycle intermediates. The maintenance of constant levels of intermediates involves continuous flux into the cycle (Spydevold et al. 1976; Lee and Davis 1979). Input of intermediates may come from the carboxylation of pyruvate and propionate (Lee and Davis 1979) or the deamination of amino acids (Lehninger 1975). All of these precursors could be derived from protein during hibernation.

Catabolism of muscle protein may provide some of the nutrient requirements of hibernation but it results in decreased muscle mass. The effect this would have on the potential for flight depends on the resulting relationship between pectoralis mass and body mass. Theory predicts that as an individual undergoes changes in body mass the power required for level flapping flight will vary with the 1.5 power of body mass (Pennycuik 1975). The difference in power requirements caused by changing body mass may be compensated by changes in kinematic parameters such as wing beat frequency and sweep angle of the wing. Compensation may also involve changes in pectoralis muscle power output deriving from changes in muscle mass. If muscle is to fully compensate for changing body mass the mass of the pectoralis muscle should vary with the 1.5 power of body mass (Marsh and Storer 1981; Pennycuik 1975). In *E. fuscus* the relationship of pectoralis mass to body mass differs through the annual body mass cycle (Fig. 3). In nonpregnant summer animals the slope (1.37) of the simple linear regression relating log pectoralis mass, $\log m_p$, to log body mass, $\log m_b$, (Eq. (1)) does not differ significantly from the predicted value (1.5) for total compensation. However, during pregnancy and prehibernation fattening the slopes of the regressions of $\log m_p$ on $\log m_b$ are significantly less than 1.5 (Eqs. (2), (3)). After four months of hibernation $\log m_p$ is not significantly correlated with $\log m_b$. This nonuniformity may result from differences between groups with respect to 1) the time course over which muscle adaptations occur and 2) the causal factor for the maintenance of a particular muscle mass. The power requirement of flight is the causal factor for the relationship between pectoralis muscle mass and body mass in active bats (Pennycuik 1975). Thus, body mass is a major determinant of pectoralis muscle mass in nonpregnant summer, pregnant, and prehibernation in bats. As a result, $\log m_p$ is strongly correlated with $\log m_b$ in each of these groups. The differences between these groups may reflect the period of time over which adaptation has occurred. The relationship between

pectoralis mass and body mass in nonpregnant summer animals develops over a relatively long period of time during which pectoralis mass and body mass change very little. This is analogous to the situation described in Cooper's hawks, *Accipiter cooperii*, (Marsh and Storer 1981) in which body mass changes slowly over a long period of time. As in the nonpregnant summer bats, changes in body mass are totally compensated by changes in muscle mass. In contrast, the changes in body mass during pregnancy and prehibernation fattening are large and occur over a period of only a few weeks. The exponents relating pectoralis mass to body mass in pregnant and prehibernation bats are significantly lower than the predicted value (1.5) for total compensation. Similarly, in the gray catbird, *Dumetella carolinensis* (Marsh 1981), muscle mass does not increase sufficiently during pre-migratory fattening to compensate fully for increased body mass. Data taken from Marsh (1979) yield the relationship:

$$\log m_p = 0.65 \log m_b - 0.36 \quad (7)$$

($r=0.73$; $P<0.001$; $n=92$). The incomplete compensation in these species may be due to physical limitations on the maximum extent of muscle enlargement possible in small flying animals. They may also be the result of a lag in muscle response. Interestingly, in the much larger species, the pied-billed grebe, *Podilymbus podiceps*, the increased body mass associated with premigratory fattening is totally compensated by increased pectoralis mass (R.L. Marsh, personal communication).

Based on the above arguments, the pectoralis muscle mass needed to support flight upon arousal of *E. fuscus* should depend on body mass upon emergence. Since body mass is significantly reduced during hibernation due to the oxidation of fat (Beer and Richards 1956; Table 1), the necessary muscle mass after four months should be less than at the outset of hibernation. However, during hibernation body mass is not a causal factor in the maintenance of a particular muscle mass. Muscle mass is determined by the rate of protein catabolism. Therefore, it is not surprising that after four months of hibernation log pectoralis mass is not significantly correlated with log body mass. However, it is remarkable that after four months of hibernation the values for pectoralis mass and body mass overlap with those of summer bats (Fig. 3; Table 2). It appears that the rate of muscle protein catabolism is such that the distribution of values for pectoralis mass and body mass after four months of hibernation is similar to that observed in nonpregnant summer bats (Fig. 3). The lack of

correlation between log pectoralis mass and log body mass in hibernating bats suggests that the maintenance of the ability to fly may be only partially dependent on the maintenance of a specific relationship between pectoralis mass and body mass. These animals are capable of flight after four months of hibernation in the laboratory. Therefore, an ability to adjust the kinematic parameters of flight such as wing beat frequency and sweep angle may play an important role in flight immediately upon arousal in the spring.

Protein is the central component of muscle, both in its contractile role and in its role as a store of gluconeogenic precursors. The changes in muscle mass during the yearly body mass cycle in *E. fuscus* are accompanied by changes in total pectoralis protein mass (Table 2). However, total pectoralis protein mass does not remain in constant relation to pectoralis muscle mass throughout the year (Table 2). The muscle enlargement occurring in prehibernation fattening differs qualitatively with that in pregnancy. The concentration of protein in the pectoralis muscles of pregnant ($248.1 \pm 4.4 \text{ mg} \cdot \text{g muscle}^{-1}$) and nonpregnant summer bats ($249.7 \pm 6.6 \text{ mg} \cdot \text{g muscle}^{-1}$) remains constant despite the large change in muscle mass (Table 2). Muscle protein concentration during prehibernation fattening ($336.1 \pm 10.9 \text{ mg} \cdot \text{g muscle}^{-1}$) is significantly greater ($P<0.001$) than in summer bats. This reflects a very large elevation of total protein mass during prehibernation fattening (Table 2; Fig. 4). During hibernation both muscle mass and muscle protein concentration decrease significantly (Table 2).

The increased protein concentration in pectoralis muscle during prehibernation fattening is puzzling. Typically, muscle hypertrophy occurs with no change in muscle protein concentration (for example, see Goldberg et al. 1975; Ianuzzo and Chen 1979). In fact, the muscle enlargement during pregnancy in *E. fuscus* does not involve changes in protein concentration (Table 2). The increased pectoralis muscle protein concentration in prehibernation bats is not due to overall dehydration (Table 1). Therefore, it appears that the protein concentration of the pectoralis muscle is preferentially increased during prehibernation fattening. Perhaps this may act as a protein store which is used during hibernation, presumably for gluconeogenesis.

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