

## ANNUAL BROWN FAT DYNAMICS IN *PIPISTRELLUS HESPERUS* AND *MYOTIS CALIFORNICUS* WITH SPECIAL REFERENCE TO WINTER FLIGHT ACTIVITY

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**Abstract**—An examination of the annual changes in brown fat deposits of *Pipistrellus hesperus* and *Myotis californicus* reveals:

1. *P. hesperus* reaches peak brown fat levels in January and February, declining rapidly through March and April, then increases gradually through the remainder of the year.
2. *M. californicus*, on the other hand, maintains peak levels of brown fat from December through February, decreasing gradually through August, then increasing rapidly in the fall.
3. The major brown fat deposits for both species are interscapular, jugular, squamooccipitocervical and carotid bodies.
4. The interscapular deposit (which accounts for 50–70% of all brown fat) appears to be a good index of brown fat dynamics.
5. Brown fat appears to be important in maintaining body temperature during flight at cold ambient temperatures.

### INTRODUCTION

The importance of brown adipose tissue in non-shivering thermogenesis is well known (Joel, 1965). Brown fat is generally used synonymously with interscapular brown fat masses, but neglects the importance of other strategically located masses. Rauch & Hayward (1969) have detailed the topography and vascularization of brown adipose tissue in *Myotis lucifugus*. Further studies have discussed the importance of brown fat bodies in differential blood flow as well as establishment of significant temperature gradients in the body of bats during arousal from hibernation (Rauch & Hayward, 1970; Rauch, 1973; Rauch & Beatty, 1975). In addition, Mason & Prychodko (1975) have discussed the changes in interscapular brown fat masses due to different thermal environments. Little is known concerning seasonal changes in brown fat mass in free-living small mammals (Buchalczyk & Korybska, 1964; Didow & Hayward, 1969). No studies have been reported on such changes in hibernating species as bats.

Several species of bats are known to be active throughout the year in cold temperate regions of the southwestern United States (O'Farrell & Bradley, 1970). These bats apparently hibernate for short periods, becoming intermittently active at reduced rectal temperatures throughout the colder months in response to declining body condition and water balance (O'Farrell & Bradley, 1977). An examination of brown fat dynamics should increase our knowledge of the phenomenon of cold weather activity in bats.

The purpose of the present study is to describe changes in brown adipose tissue mass throughout the year for *Pipistrellus hesperus* and *Myotis californicus*. These changes are examined in relation to known thermal strategies, and possible physiological advantages are discussed.

### MATERIALS AND METHODS

The majority of the bats examined were captured in "mist nets" at White Spot Spring, Desert National Wildlife Range, Clark County, NV between 1962 and 1967. In a few instances, samples were low or non-existent; therefore, to complete the annual cycle, required specimens were obtained at Grapevine Spring, Spring Mountains, Clark County, NV in 1976. All animals were removed from the net immediately, placed in holding sacks and returned to the laboratory where they were preserved in 10% formalin and stored in 70% ethanol.

Brown fat deposits were dissected out, blotted dry, and weighed to the nearest 0.1 mg on a Mettler balance. The specific deposits we were able to weigh were as follows: interscapular, jugular, squamooccipitocervical, carotid, clavoscapular, subscapular, basioccipital and pericardial. Identification and nomenclature follows Rauch & Hayward (1969). Other brown fat deposits were too minute to weigh. All weights were converted to grams/kilogram live body weight.

### RESULTS

Annual cycles for total brown fat differed between the two bat species (Figs 1 and 2). Data were analysed by sex but no sexual differences were observed. The variability within samples for late winter was high for both species and they both demonstrate a decline in brown fat mass with a subsequent autumn deposition. However, the decrease in *P. hesperus* brown fat was precipitous, occurring in early spring. A progressive increase occurred from mid-spring (April) into the winter. In contrast, *M. californicus* brown fat declined progressively from winter to August, at which time fat deposits increased rapidly through the fall.

The contribution of the major brown fat deposits to the total is presented for *P. hesperus* and *M. californicus* (Tables 1 and 2, respectively). The interscapular

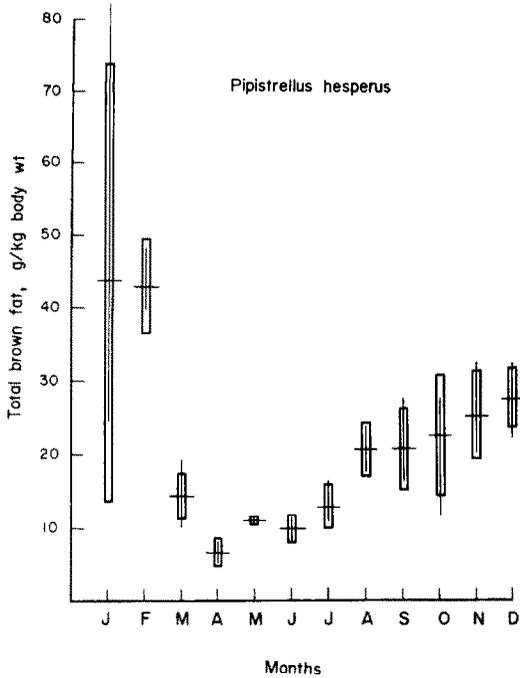


Fig. 1. The cycles of total brown fat (g/kg body wt) for *Pipistrellus hesperus*. Vertical bar, range; horizontal bar, mean; enclosed box,  $\pm 2$  S.E.

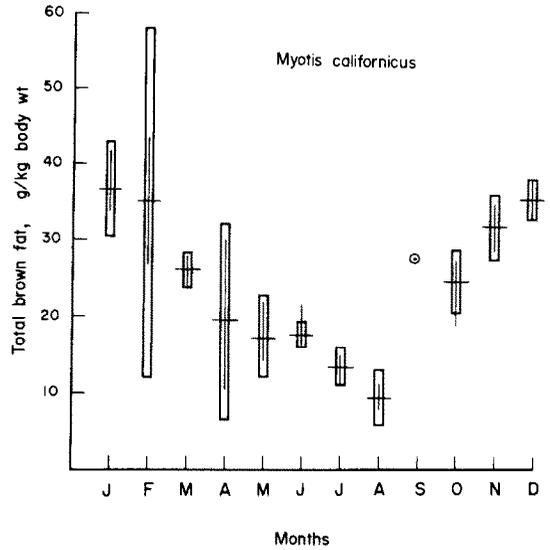


Fig. 2. The cycles of total brown fat (g/kg body wt) for *Myotis californicus*. Vertical bar, range; horizontal bar, mean; enclosed box,  $\pm 2$  S.E.

deposit accounts for 50% or more of total brown fat and follows the cycles shown in Figs 1 and 2. It appears that this deposit can be used reliably to reflect total brown fat cycles in bats. The jugular deposit in *P. hesperus* shows an abrupt drop from February to March, little change from March through July, and then a slow increase during the rest of the year (Table 1). The jugular deposit in *M. californicus* follows the same pattern as the interscapular deposit (Table 2, Fig. 2). The squamooccipitocervical deposit

in both species shows high variability in January and February but little change through the rest of the year. Similarly, the carotid deposit for both species shows virtually no change throughout the year except for one exceptionally fat *P. hesperus* in January (Tables 1 and 2).

DISCUSSION

Several studies have dealt with the seasonal changes in mass of brown adipose tissue in two species of small mammals, the shrew, *Sorex araneus* (Buchalczyk & Korybska, 1964) and the meadow vole, *Microtus pennsylvanicus* (Didow & Hayward, 1969). Neither of these species hibernates but remain

Table 1. Mean brown fat weights (g/kg body weight) for the four largest fat deposits and total brown fat deposits for *Pipistrellus hesperus*

Month	N	Interscapular	Jugular	Squamo-occipito-cervical	Carotid	Total
January	4	21.02 (8.20)	10.22 (6.14)	4.02 (4.04)	3.69 (6.48)	43.81 (30.04)
February	3	22.99 (12.12)	13.09 (5.76)	4.02 (3.34)	1.70 (0.82)	43.08 (6.38)
March	5	10.60 (2.70)	2.60 (0.90)	0.37 (0.22)	0.30 (0.20)	14.48 (3.08)
April	3	3.44 (2.22)	1.15 (0.92)	1.26 (1.18)	0.51 (0.14)	6.56 (1.82)
May	2	5.99 (1.42)	2.66 (1.28)	1.56 (0.02)	0.65 (0.18)	11.29 (0.02)
June	4	6.10 (2.12)	2.00 (0.10)	0.53 (0.34)	0.88 (0.44)	9.94 (1.76)
July	4	8.10 (2.40)	2.08 (0.54)	1.20 (1.16)	0.68 (0.18)	13.13 (2.90)
August	4	10.55 (3.22)	5.43 (2.56)	1.10 (0.28)	1.00 (0.28)	20.84 (3.68)
September	4	12.45 (3.24)	5.28 (2.50)	1.63 (1.86)	0.60 (0.16)	20.92 (5.48)
October	4	13.13 (4.52)	5.30 (2.92)	1.38 (0.48)	0.83 (0.22)	22.75 (8.30)
November	4	13.65 (3.56)	6.70 (1.72)	1.70 (0.48)	0.93 (0.16)	25.46 (5.98)
December	4	15.58 (3.26)	7.28 (0.94)	1.85 (1.20)	1.28 (0.72)	27.83 (3.98)

2 S.E.<sub>x</sub> are given in parentheses.

Table 2. Mean brown fat weights (g/kg body weight) for the four largest fat deposits and total brown fat deposits for *Myotis californicus*

Month	N	Interscapular	Jugular	Squamo-occipito-cervical	Carotid	Total
January	3	21.30 (6.24)	7.27 (1.34)	3.29 (2.34)	1.90 (0.86)	36.68 (6.26)
February	2	19.70 (10.46)	8.35 (3.80)	4.43 (8.86)	1.44 (0.96)	35.17 (23.16)
March	4	16.50 (2.22)	4.50 (0.68)	1.60 (0.40)	1.13 (0.38)	26.09 (2.38)
April	3	10.35 (6.46)	4.12 (3.94)	2.37 (2.18)	1.61 (0.04)	19.61 (13.12)
May	3	7.84 (3.78)	2.85 (0.52)	3.98 (1.04)	1.83 (0.50)	17.48 (5.26)
June	6	8.83 (1.54)	2.67 (0.92)	1.97 (0.04)	1.92 (0.10)	17.71 (1.66)
July	3	7.33 (2.16)	1.77 (0.30)	1.53 (0.60)	1.80 (0.50)	13.43 (2.46)
August	2	4.00 (2.82)	1.60 (1.14)	1.10 (0.28)	1.65 (0.42)	9.46 (3.64)
September	1	18.00	6.00	0.90	1.10	27.88
October	4	16.25 (2.77)	3.65 (1.44)	1.28 (0.42)	1.18 (0.56)	24.71 (4.20)
November	3	19.00 (1.42)	8.00 (2.44)	1.30 (0.62)	1.17 (0.60)	31.87 (4.16)
December	4	20.00 (0.00)	9.25 (2.38)	1.78 (0.24)	1.78 (0.38)	35.47 (2.70)

2 S.E.<sub>x</sub> are given in parentheses.

active throughout the year in microclimates consistently below the thermoneutral zone. Didow & Hayward (1969) demonstrated the adaptive value of autumn deposition of brown fat and explained the cycle of deposition and mobilization as a response to changing microclimate. Since these animals do not require a thermogenic arousal stimulus it was proposed that the brown adipose tissue was functional in non-shivering thermogenesis, maintaining a constant body temperature at low microclimate temperatures.

The bat species examined in the present study are found actively flying throughout the year in southern Nevada (O'Farrell & Bradley, 1970) but appear to hibernate for short periods throughout the winter months (O'Farrell & Bradley, 1977). Brown fat is an important heat source during arousal from hibernation (Hayward & Lyman, 1967); thus we would expect heavy depletion pressure on the fat deposits of these bats. An examination of Figs 1 and 2 does not reveal rapid depletion during the winter in either species, a condition probably due to opportunistic feeding on warmer nights throughout the winter (O'Farrell & Bradley, 1970), which would account for the abrupt decline in brown fat deposits in *P. hesperus* (Fig. 1). A similar trend is not evident for *M. californicus*, which has greater insulation and would not be under as much thermal stress during activity (O'Farrell & Bradley, 1977). It should be stressed that the bats examined in this study were captured while actively flying over waterholes and would represent those individuals actually forced into activity due to reduced body conditions and negative water balance. We therefore speculate that the individuals in hibernation at this time would have higher fat stores.

Didow & Hayward (1969) question whether the interscapular deposit is an adequate index of total brown fat changes. They found that for *Microtus pennsylvanicus* the interscapular deposit accounts for less than 40% of the total brown fat tissue. We feel that, at least for the two bat species in the present study, interscapular brown fat is a good index of total brown fat changes. The interscapular mass accounts for approximately 50–70% of the total throughout the year (Tables 1 and 2).

The periodic flights throughout the winter to obtain water and occasional food (O'Farrell & Bradley, 1977) raises another question concerning the maintenance of body temperature at low ambient temperature. Both species range in weight from 2.5 to 5 g, possess a large, naked, highly vascularized surface area, and have been observed flying at ambient temperatures as low as  $-8^{\circ}\text{C}$  (O'Farrell & Bradley, 1970). One mechanism the bats use to accommodate to such stressful situations is to reduce the thermal gradient between body and ambient temperature by operating at reduced body temperatures (Bradley & O'Farrell, 1969; Studier & O'Farrell, 1972; O'Farrell & Bradley, 1977). This is accomplished in part by establishing a thermal gradient between the anterior and posterior portions, as well as central and peripheral portions of the body utilizing differential blood flow, as shown for arousing bats by Rauch & Hayward (1970), Rauch (1973), and Rauch & Beatty (1975). Hirshfeld & O'Farrell (1976) discuss topographical differences in body temperature.

It is reasonable to assume that brown adipose tissue is responsible for the initial arousal thermogenesis for periodic winter flights. Once body temperature reaches the level where flight may be achieved the problem then becomes the maintenance of that temperature under conditions of high convective heat loss. The energy required for temperature maintenance can come from increased metabolic activity, mechanical heat production from flight muscle contraction, and from brown fat metabolism. Under stressful winter conditions we suspect that all three sources are utilized.

Brown fat vascular relationships have been reviewed by Rauch & Hayward (1969, 1970), Rauch (1973) and Rauch & Beatty (1975). During arousal, blood distribution is restricted until late in arousal; consequently, the cervical and thoracic regions warm up much faster. Presumably this regional increase in temperature is a reflection of increased brown fat metabolism and the regionalized blood circuits in the cervico-thoracic region. In addition to the major brown fat bodies, there are smaller accumulations associated with several of the internal organs of the thoraco-abdominal region. These deposits, while too small to supply appreciable quantities of heat to the vascular system for redistribution, probably provide sufficient heat to activate adjacent organs (e.g. renal and pericardial deposits). The most significant transfer of heat from brown fat, however, is that which occurs to the blood (Rauch & Hayward, 1969).

The network of brown adipose tissue in relation to blood flow suggests an efficient heating system for critical organs and flight muscles. As blood flows to wing muscles and to the head it loses heat but is warmed rapidly as it returns to the body core. Rauch (personal communication) has failed to find a counter-current system in the wings or wing muscles. A countercurrent system would not be as efficient as the brown fat warming system. We feel as a recommendation that thermal mapping during low temperature flight is needed in order to elucidate the role of brown adipose tissue in the maintenance of a stable body temperature.

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#### REFERENCES

- BRADLEY W. G. & O'FARRELL M. J. (1969) Temperature relationships of the western pipistrelle (*Pipistrellus hesperus*). In *Physiological Systems in Semi-arid Environments* (edited by HOFF C. C. & REIDSESEL M. L.), pp. 85–96. University of New Mexico Press, Albuquerque.
- BUCHALCZYK A. & KORYBSKA Z. (1964) Variations in the weight of brown adipose tissue of *Sorex araneus* Linnaeus, 1758. *Acta theriol.* **14**, 193–215.
- DIDOW L. A. & HAYWARD J. S. (1969) Seasonal variations in the mass and composition of brown adipose tissue in the meadow vole, *Microtus pennsylvanicus*. *Can. J. Zool.* **43**, 547–555.
- HAYWARD J. S. & LYMAN C. P. (1967) Non-shivering heat production during arousal from hibernation and evidence for the contribution of brown fat. In *Mammalian*

- Hibernation*, Vol. 3 (edited by FISHER K. C., DAWE A. R., LYMAN C. P., SCHONBAUM E. & SOUTH F. E. JR), pp. 346-355. American Elsevier, New York.
- HIRSHFELD J. R. & O'FARRELL M. J. (1976) Comparisons of differential warming rates and tissue temperatures in some species of desert bats. *Comp. Biochem. Physiol.* **55A**, 83-87.
- JOEL C. D. (1965) The physiological role of brown adipose tissue. In *Handbook of Physiology*, Sec. 5, *Adipose Tissue* (edited by REYNOLDS A. E. & CAHILL J. F.), pp. 59-86. American Physiological Society, Washington.
- MASON E. B. & PRYCHODKO W. (1975) Interscapular brown adipose tissue mass in two species of *Peromyscus* raised in different thermal environments. *J. Mammal.* **56**, 683-685.
- O'FARRELL M. J. & BRADLEY W. G. (1970) Activity patterns of bats over a desert spring. *J. Mammal.* **51**, 18-26.
- O'FARRELL M. J. & BRADLEY W. G. (1977) Comparative thermal relationships of flight for some bats in the southwestern United States. *Comp. Biochem. Physiol.* **58A**, 223-227.
- RAUCH J. C. (1973) Sequential changes in regional distribution of blood in *Eptesicus fuscus* (big brown bat) during arousal from hibernation. *Can. J. Zool.* **51**, 973-981.
- RAUCH J. C. & HAYWARD J. S. (1969) Topography and vascularization of brown fat in a hibernator (little brown bat, *Myotis lucifugus*). *Can. J. Zool.* **47**, 1315-1323.
- RAUCH J. C. & HAYWARD J. S. (1970) Regional distribution of blood flow in the bat (*Myotis lucifugus*) during arousal from hibernation. *Can. J. Physiol. Pharmacol.* **48**, 269-273.
- RAUCH J. C. & BEATTY D. D. (1975) Comparison of regional blood distribution in *Eptesicus fuscus* (big brown bat) during torpor (summer), hibernation (winter), and arousal. *Can. J. Zool.* **53**, 207-214.
- STUDIER E. H. & O'FARRELL M. J. (1972) Biology of *Myotis thysanodes* and *M. lucifugus* (Chiroptera: Vespertilionidae)—I. Thermoregulation. *Comp. Biochem. Physiol.* **41A**, 567-595.