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SEASONAL CHANGES IN BROWN FAT AND PELAGE IN SOUTHERN SHORT-TAILED SHREWS

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We examined cellular changes in interscapular brown adipose tissue and pelage characteristics in *Blarina carolinensis* collected throughout the year in eastern Virginia. Cellular volume occupied by mitochondria and maximum mitochondrial size were significantly greater in the brown adipose tissue of winter shrews than in summer shrews. Lipid droplets occupied greater volume and were larger in shrews in summer than winter shrews. There were no seasonal differences in hair density; Type I and Type II guard hairs were significantly longer in winter than summer by a factor of 1.3. Woolly hairs were 1.2 times longer in winter than summer, a non-significant difference.

Key words: *Blarina carolinensis,* short-tailed shrew, brown adipose tissue, pelage, mitochondria

Blarina carolinensis, the southern short-tailed shrew, like other small non-hibernating mammals, must adapt to survive winter conditions. Southern short-tailed shrews are small (adults 7–10 g) with short pelage, so they rapidly lose heat due to their large ratio of surface area to body volume. To survive winter, *B. carolinensis* relies in part on an increase in cold-induced thermogenesis, resulting in a type of facultative thermogenesis that is initiated according to circumstance. These increases in heat production can result from shivering or nonshivering thermogenesis (Himms-Hagen, 1985, 1986). Numerous studies on nonshivering thermogenesis in small mammals show that it takes place in brown adipose tissue (Bukowiecki et al., 1982; Didow and Hayward, 1969; Girardier, 1983; Himms-Hagen, 1986; Merritt, 1986). Brown adipose cells, primarily found in the interscapular region, contain many large rounded pleiomorphic mitochondria with tightly packed cristae (Bukowiecki et al., 1982; Cannon and Nedergaard, 1985; Fawcett, 1952; Girardier, 1983; Hyvärinen, 1994; Lindberg et al., 1967; Rothwell and Stock, 1985; Slavin, 1987). During cold exposure, a significant increase in the uncoupling protein, thermogenin, enables the mitochondrial inner membrane to have the high proton permeability that renders the mitochondria thermogenic, thus producing heat at a rate of 300 watt/Kg of brown adipose tissue (Cannon and Nedergaard, 1987).

Both the level of nonshivering thermogenesis and the relative amount of brown adipose tissue in small mammals vary inversely with ambient temperature (Didow and Hayward, 1969; Merritt, 1986; Merritt and Zegers, 1991; Zegers and Merritt, 1988*a*, 1988*b*). The studies of Merritt and Zegers of *Blarina brevicauda, Peromyscus maniculatus, Peromyscus leucopus* and *Clethrionomys gapperi* led them to suggest that photoperiod and ambient temperature interact to trigger changes in nonshivering thermogenesis. Hyvärinen (1994) found that the relative amount of brown adipose tissue in winter shrews from Finland was related inversely to body mass; highest relative weights of brown adipose tissue were found in the smallest species. High proportions of brown adipose tissue enable small mammals to thermoregulate and survive winter conditions in north temperate and subarctic locations.

Small mammals can minimize their en-

ergy needs and expenditures by increasing both density and length of hairs during winter, thus minimizing thermal conductance. Thermal conductance is 27.6% higher in winter than summer in *Blarina brevicauda* (Bozinovic and Merritt, 1992). *Microtus agrestis* (Al-Khateeb and Johnson, 1971*a*, 1971*b*) has denser hair in winter than in summer due to both groups of hair follicles being closer together and an increase in number of follicle groups, with a larger increase in number of fine hairs than guard hairs. Guard hairs also increase in width from summer to winter. Findley (1956) found that winter pelage of *B. brevicauda* is distinguished from summer pelage by greater length and density of fur. In a detailed pelage study of four soricine shrews from extreme western Russia, Ivanter (1994) found that Type I guard hairs, Type II guard hairs, and woolly hairs all increase in length by 30–40% in winter. Further, although hair density increased in winter, density was greatest during autumnal and vernal molts (when both old and emerging hairs were present).

Our first objective was to quantify microanatomical changes in brown adipose tissue throughout the year for wild-caught *B. carolinensis*. Merritt (1986) observed a strong seasonal relationship between amounts of brown adipose tissue and heat production by nonshivering thermogenesis in *B. brevicauda* in a natural setting. Because mitochondria are needed in winter to produce large amounts of heat by nonshivering thermogenesis, we hypothesized a seasonal change in volume of mitochondria in adipocytes of brown adipose tissue. Because heat production probably is related to volume of mitochondria, we expected to see the greatest differences between shrews collected in winter and summer, with intermediate values from shrews collected in spring and autumn.

Previous studies have dealt with laboratory species exposed to artificial acclimation. For example, Suter (1969) observed that mitochondria in cold-exposed rats occupied a greater area of the cytoplasm and were packed more densely and flattened against each other than those in rats raised at room temperatures. Lipid droplets in cold-exposed rats decreased in size compared to those in rats raised at room temperature, while number of small droplets increased after prolonged exposure to low temperature. We hoped that, by examining tissues collected throughout the year, we would see changes in percentages of volume and relative sizes of mitochondria and lipid droplets and thus see the pattern of seasonal changes.

Our second objective was to determine if *B. carolinensis* undergoes pelage changes to cope with temperature changes. Seasonal comparisons were made of hair length, number of segments in the hairs, and hair density in the interscapular regions of the animals, above the area of interscapular brown adipose tissue. We hypothesized a seasonal difference in both the hair density and hair length based on previous studies on shrews. Ivanter (1994) reported a 20–30% increase in the pelage density of four species of soricids as winter approached in western Russia. That change in density was due to an increase in number of woolly hairs, which are used more for insulation than for protection. However, Ivanter (1994) observed the highest densities during molting in autumn and spring. Therefore, we expected to observe significantly higher densities of hairs during molting in autumn and spring. *B. brevicauda* in Kansas has its spring molt between February and July and its autumn molt in October or November (Findley, 1956). Because *B. carolinensis* is closely related, we expected greater hair densities during these months of molt.

Ivanter (1994) found that winter hairs were 1.8–2.0 times longer than summer hairs and the number of segments increased from 3–4 to 6–7 in four species of soricids in western Russia. Thus, we expected to find both longer hairs and an increase in

number of segments of hairs from shrews collected in winter.

Materials and Methods

Seventy-four *B. carolinensis* of both sexes were collected at monthly intervals between January and December, 1991 under a trapping permit issued by the Commonwealth of Virginia. Shrews were taken from a shrub-forb site in Portsmouth, Virginia using Fitch live traps. Each Fitch trap was constructed of galvanized wire mesh with a swinging treadle, gravity-operated drop door, and an aluminum drink can (ca. 340 ml) for a nest box (Rose, 1994). About 90 traps, baited with sunflower seeds, were set in two or three sections of a regenerating old field. Each trap contained polyester fiber for bedding. Traps were set in the late afternoon and were checked after dusk (ca. 2000–2200 h) and again in early morning (0700 h). Shrews that were found alive were used for the study of brown adipose tissue, whereas those that died in the traps, when no time of death could be established, were used for hair analysis.

Up to 48 h after capture and following regulation protocols of ethical care and treatment of animals, live shrews were anesthetized lightly with ether and then injected intraperitoneally with 0.1–0.2 ml of 2.5% tribromo-ethanol, a treatment which induced deep anesthesia. Shrews then were perfused via the left ventricle with a buffered solution of 2.5% glutaraldehyde and 2% formaldehyde. Following immersion in the same fixative for 12–24 h, interscapular brown adipose tissue was dissected out and, to avoid contamination with salivary and lacrimal glands which are found in close proximity, only middle sections of tissue were processed for study by transmission electron microscopy. Tissue was rinsed in buffer, placed in 2% buffered osmium tetroxide for 3 h, and embedded in epoxy resin (Polybed 812, Polysciences, Inc., Warrington, PA) for sectioning at a thickness of 70–90 nm.

A JOEL 100 CXII transmission electron microscope (JOEL USA, Peabody, MA) was used to examine cellular components of adipose tissue. Four of 7–10 micrographs from each sample randomly were chosen for analysis of percentages of cellular volume occupied by each structure in each season.

To minimize investigator bias, a four-quadrant grid for each micrograph was used. The grid was placed in the middle of the micrograph, and one quadrant was selected randomly by drawing lots. In each quadrant, areas of mitochondria, lipid droplets, and nuclei were measured to compute total area occupied by each in the cell. All measurements were made by the same investigator using an IBM PC/AT microcomputer, a digitizing tablet, and SigmaScan software (Jandel, San Rafael, CA).

Micrographs were placed on the tablet, and each structure was outlined using a cursor with a crosshair. An image was produced on the screen as each structure was outlined by the tracer puck, thereby eliminating the possibility of remeasuring the same mitochondrion or lipid droplet. Each micrograph (ranging from 9,720× to 14,400×) was individually calibrated to compensate for differences in magnification. Total area occupied by each structure and maximum sizes of mitochondria and lipid droplets in each micrograph were calculated. Percentages of total cell area occupied by mitochondria and lipid droplets were calculated by dividing total organelle area by the quadrant area, minus blood vessel area, thereby giving percentages per cell type for each micrograph. The Delesse principle (Weibel and Bolender, 1973) states that relative area on sections is an unbiased estimate of relative volume of structures. By tracing area of all structures contained in the section, adding areas and dividing by area of the section, we calculated volume densities for both mitochondria and lipid droplets. Due to the high magnification, a micrograph quadrant usually contained one cell or parts of two cells.

A Student's *t*-test with a significance level of 0.05 was used to compare morphological differences of tissues from shrews collected in the extreme seasons of winter (December–February) and summer (June–August). This was followed with a Student's *t*-test for the intervening seasons. For percentages of cellular volume occupied by the structures, data were transformed for analysis by arc-sin^2 transformation to best fit assumptions of the *t*-test.

Electron micrographs were made of skins of shrews that died in traps. Skins were pinned flat and shaved. Small sections were cut from the interscapular region, an area of little wear. They were marked into a 1-mm^2 grid, and immersed in osmium tetroxide for 2 h. Sections were dried with liquid carbon dioxide using a DCP1 critical point dryer (Denton Vacuum, Inc., Cherry Hill,

TABLE 1.—*Seasonal differences in brown adipose tissue in* Blarina carolinensis *in Virginia as shown by mean size and relative cellular volume of mitochondria and lipid droplets, and by number of lipid droplets, as determined by transmission electron microscopy. Statistical values show a comparison of means for summer and winter specimens using a Student's* t*-test with a significance level of 0.05.*

		Mitochondria		Lipid droplets		
	n	Relative volume (%)	Maximum size (μm)	Relative volume (%)	Maximum size (μm)	Number per cell
Winter	4	20.5	2.262	41.7	25.140	8.00
Spring	4	9.6	2.632	65.4	42.250	8.25
Summer	4	5.7	1.408	81.0	58.751	6.82
Autumn	4	16.8	2.053	56.5	52.480	4.50

NJ) to prevent distortion due to air drying, and then sputter-coated with gold/palladium alloy to ensure electrical conductivity (Cohen, 1974) using an E5200 sputter-coater (Biorad, Cambridge MA). Skins were viewed with a Cambridge S100 scanning electron microscope (Leo Electron Microscopy, Inc., Cambridge, UK), and micrographs were made of individual grids at ca. 60×. An average count was determined for each individual using three subsamples of hair follicles from shrews captured in each of the four seasons.

To determine length and segmentation, we used a micrometer eyepiece to view individual hairs that had been removed from skins. Hairs were separated into three width categories (Type I guard, Type II guard, and woolly—Ivanter, 1994). We measured ca. 10 hairs of each type from shrews chosen randomly from each season and calculated mean length for each hair type from each individual. Hair density, hair length, and number of segments were analyzed with a model I analysis of variance (ANOVA), at a significance level of 0.05, followed by a Ryan-Einot-Gabrial-Welsch Multiple Comparison test, with samples from the four seasons (SAS Institute, Inc., 1990).

RESULTS

Both mitochondria and lipid droplets differed significantly in morphology between shrews collected in winter and summer (Table 1). Mitochondria of brown adipose tissue of shrews collected in winter (Fig. 1a) were significantly larger and occupied a significantly greater cellular volume (mitochondrial cellular volume—$t = 3.251$, $P = 0.044$; maximum mitochondrion size—$t = 3.239$, $P = 0.018$) than those from shrews collected in summer (Fig. 1c). However, mitochondria of shrews in summer appeared to be more electron-dense with cristae that were less well-defined than those in other seasons. Mitochondria of brown adipose tissue collected from shrews in both spring (Fig. 1b) and autumn (Fig. 1d) occupied volumes intermediate to those of summer and winter; maximal size of mitochondria was slightly, but not significantly, larger in spring (mitochondrial cellular volume—$t = 0.635$; $P = 0.569$).

Lipid droplets occupied significantly greater cellular volumes in summer than winter ($t = 8.939$, $P < 0.001$). This increase was due to an increase in droplet size and not an increase in number of droplets ($t = 0.434$, $P = 0.693$). Tissues from shrews collected in summer showed somewhat fewer but significantly larger lipid droplets, whereas tissues from shrews collected in winter showed larger numbers of small lipid droplets. Tissues from shrews collected in both spring and autumn had lipid droplets that were intermediate in size and occupied intermediate cellular volumes to those from winter and summer shrews. As with mitochondria, autumnal values were not significantly different from spring values (lipid cellular volume—$t = 0.996$, $P = 0.356$; maximum lipid droplet size—$t = 0.447$, $P = 0.670$).

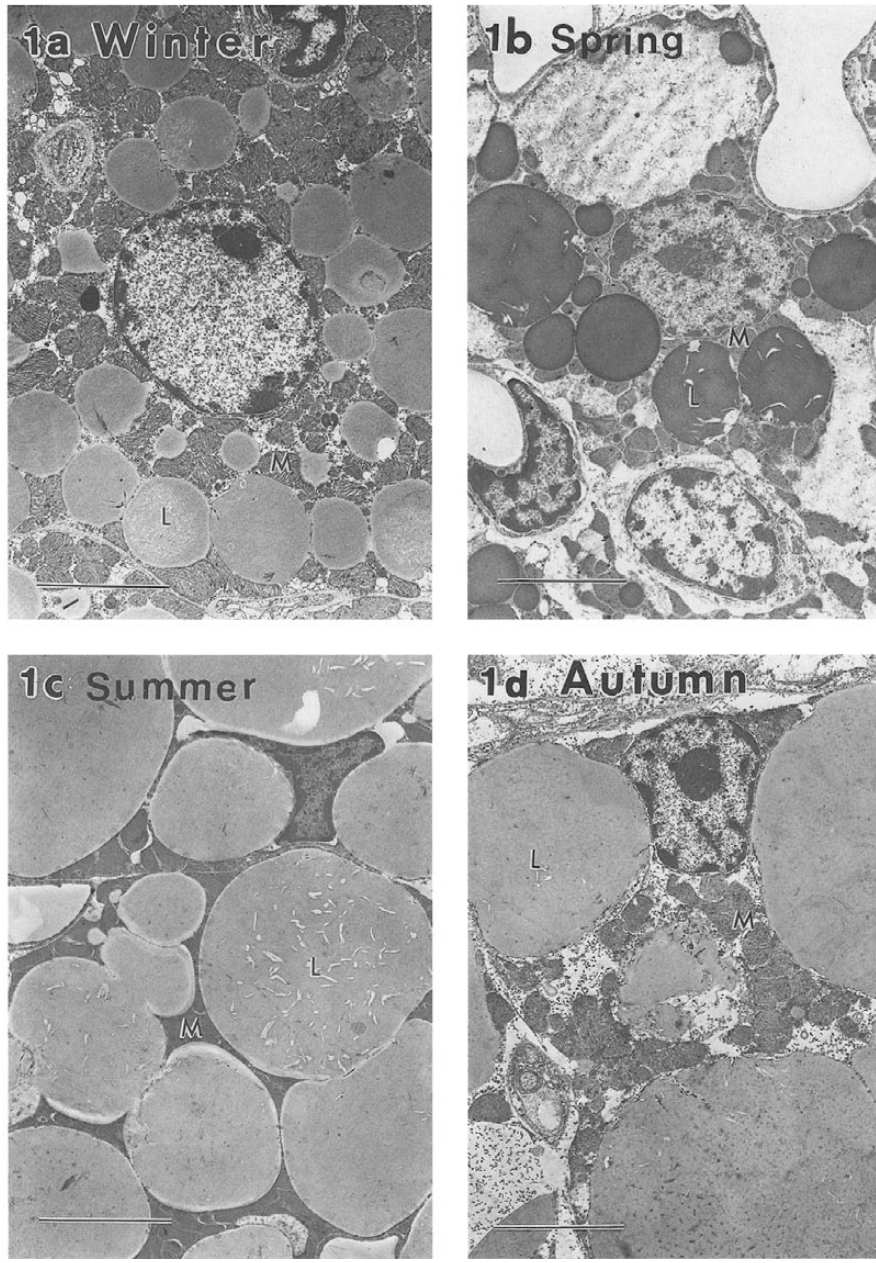

FIG. 1.—Mitochondria and lipid droplets in brown adipose tissue as seen by electron microscopy (4,320×, bar = 5 μm). a) Brown adipose tissue from a shrew collected in February, showing the large volume occupied by mitochondria (M), which have well-defined cristae. Note the relatively small lipid droplets (L) and the nucleus (N). b) Brown adipose tissue from a shrew collected in April, showing the decrease in volume of mitochondria (M) and an increase in size of lipid droplets (L) as compared to tissue from shrews collected in the winter. c) Brown adipose tissue from a shrew collected in July, showing the reduction of volume occupied by mitochondria (M) in the presence of large lipid droplets (L). d) Brown adipose tissue from a shrew collected in November showing similar volume density of mitochondria (M) as the tissue from shrews collected in the spring. The lipid droplets (L) are intermediate in size between those of shrews collected in summer and winter.

TABLE 2.—*Pelage density and hair lengths as determined by scanning electron microscopy in* Blarina carolinensis *in four seasons in Virginia. Measurements and segment counts were made on pelages of four shrews in each season (hair density—three subsamples per shrew, hair length—10 subsamples per shrew). Statistical values show a comparison of means for the four seasons using a Model I ANOVA with a significance level of 0.05.*

	Pelage density (hairs/mm²)		Hair lengths (μm)						Mean number of segments		
			Guard hairs								
			Type I		Type II		Woolly hairs		Guard hairs		Woolly
	$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*	Type I	Type II	hairs
Winter	132.5	14.4	5,702.1	121.1	5,176.7	190.3	4,719.8	45.4	5.2	5.0	5.4
Spring	135.8	10.1	5,233.8	357.6	4,938.4	125.3	4,429.8	265.2	4.7	5.2	5.2
Summer	168.3	8.4	4,363.3	201.8	3,936.3	212.6	3,828.1	175.3	4.0	4.8	4.8
Autumn	155.3	11.9	5,318.2	271.4	4,844.1	285.1	4,634.3	294.9	5.0	5.2	5.2

There were no seasonal differences in hair density ($F = 2.40$, $P = 0.119$), but lengths of Type I ($F = 3.40$, $P = 0.004$) and Type II ($F = 6.62$, $P = 0.006$) guard hairs showed significant seasonal differences (Table 2). In both cases, winter hairs were 1.3 times longer than summer hairs. Differences in lengths of woolly hairs were marginally nonsignificant between seasons ($F = 3.40$; $P = 0.054$).

Compared to shrews collected in summer, only Type I guard hairs ($F = 3.14$, $P = 0.048$) of shrews collected in winter had significantly more hair segments. Neither Type II guard hairs ($F = 1.22$, $P = 0.344$) nor wooly hairs ($F = 1.6$, $P = 0.229$) showed significant differences in numbers of hair segments across seasons. Hairs of shrews collected in spring and autumn were not significantly different than those from winter or summer.

DISCUSSION

There were substantial seasonal morphological changes in brown adipose tissue of *B. carolinensis*. Cytoplasmic features of brown adipose cells changed in a manner consistent with seasonal responses in nonshivering thermogenesis in *B. brevicauda* (Merritt, 1986). In the winter, cells had almost four times greater volume of mitochondria than in summer (Table 1), which would give cells greater thermogenic potential. Mitochondria of winter adipocytes were 1.6 times as large as in summer. These differences in mitochondrial size correspond with the results of Lindgren and Barnard (1972), who found that mitochondria of brown adipose tissue in rats are most developed for oxidative capacity after cold acclimation.

Our results support those of Suter (1969), who reported that mitochondria of cold-acclimated rats (*Rattus*) occupy a greater area of the cytoplasm and they spread apart with straighter and more parallel cristae than in control rats. He also found that the ratio of cristae to matrix increased with cold treatment.

Interscapular brown adipose tissue showed an inverse relationship between cellular volume occupied by mitochondria and cellular volume occupied by lipid droplets. The volume occupied by lipid droplets nearly doubled from winter to summer, suggesting fat use in winter and fat storage in summer, whereas the volume occupied by mitochondria decreased by a factor of nearly four from winter to summer (Table 1). In transitional seasons (spring and autumn), values were intermediate. This is in agreement with Suter (1969), who reported that after cold-acclimated rats were placed again at 22°C, there was a reversal of the cold-induced changes with mitochondria decreasing in size and number.

Because *B. carolinensis* is not a hibernator, it probably relies heavily on nonshivering thermogenesis for winter survival. As summer approaches the need for nonshivering thermogenesis decreases; thus, fewer mitochondria are needed for heat production. There is a gradual decrease in volume of mitochondria as their numbers and sizes decline to minimal values. Meanwhile, the amount of lipid increases because energy that had been required for maintenance can now be stored, perhaps in anticipation of future demands of reproduction. It is possible that the small lipid droplets, seen in winter, coalesce into larger droplets in summer and then break apart again for easier metabolism as winter approaches.

Although our morphological results were similar to the findings of Loncar et al. (1988) and Suter (1969), we found inconsistencies. One shrew in winter had a mitochondrial volume density of only 6.7% compared to an average of 25% for the three others. Tissue from that shrew had large intracellular spaces filled with what appeared to be glycogen and lipid droplets that were larger than those observed in other winter shrews. Thus, there is some variation in cellular composition that remains to be understood.

Winters are mild in eastern Virginia and very mild in relation to winters in northern areas of the distributional range of *Blarina*. Temperatures <0°C are recorded only a few nights each winter, and the lowest temperature during the study period was −2.2°C in February. No measurable snow fell in 1991. We predict that the seasonal contrast in mitochondrial and lipid cellular volumes will be greater in *Blarina* from regions with more extreme temperatures.

Lack of seasonal differences in hair density did not support our hypothesis, which was based on previous studies that reported differences in density of hair (Ivanter, 1994). In contrast, seasonal differences in hair length did correspond to previous studies on *Sorex* (Ivanter, 1994). All three segmented hair types were 1.2–1.3 times longer in the winter than summer in *B. carolinensis*. However, only Type I guard hairs increased in segmentation, from 3–4 segments in summer to 5–6 segments in winter.

A dense pelage of longer hairs would be adaptive by reducing thermal conductance in winter. Bozinovic and Merritt (1992) found that thermal conductance was 27.6% lower during winter than summer in *B. brevicauda* from Pennsylvania. This reduction in conductance would reduce energy expenditure during thermoregulation. *B. carolinensis* showed significant increase in hair length to cope with winter but no significant changes in density of insulative (woolly) hairs. Perhaps the observed density was influenced by the mild winter conditions; *Blarina* farther north likely will show stronger differences in density of winter hairs.

In conclusion, significant morphological changes in mitochondria in relation to season support the findings of others and lend support to the observation that capability for nonshivering thermogenesis is greatest in winter. Significant changes in sizes of lipid droplets and changes in relative cellular volume suggest that lipid content of brown adipose tissue also is directly related to ambient temperature. Occurrence of shorter Type I and Type II guard hairs in summer supports the findings of others, but unexpectedly, there was no significant change in hair density in response to season in *Blarina* from eastern Virginia.

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