AN ABSTRACT FOR THE THESIS OF

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Title: Seasonal variation in hematology, body composition, and food caches of eastern woodrats (Neotoma floridana)

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Winter is a critical time for any small mammal in a seasonal environment due to the increased energy demands for thermoregulation and decreased food availability. Small mammals make behavioral and physiological adjustments in order to survive the winter. One such behavioral adjustment is that of a larder hoarder that stores a centrally located food cache, which serves as an energy supply during times of resource scarcity. Small mammals have been shown to make hematological adjustments to increase oxygen carrying capacity in the winter as well. I studied seasonal changes in the hematology (including serum chemistry), body composition, and food caches of eastern woodrats (Neotoma floridana) in east-central Kansas from October 1999 to August 2000. I also evaluated the use of total body electrical conductivity (TOBEC) as a method for estimating lean and lipid mass in eastern woodrats.

Hematological analysis showed that packed cell volume (PCV) and red blood cell (RBC) counts were highest in the winter and lowest in the summer, which was consistent with patterns that had been observed previously in other small mammals. However, hemoglobin (HB) levels were lowest in the winter, which was suggestive of iron-deficiency anemia. Plasma lipid levels were lowest in the winter and plasma protein concentrations were constant throughout the seasons studied. Percent body fat was lowest in the winter and highest in the spring and the total energy of food caches was highest in the winter and lowest in the spring. Fall and spring showed intermediate levels in hematological values. The increased PCV and RBC count of wintering eastern woodrats was likely in response to increased metabolic activity for thermoregulation. Eastern woodrats also showed a relationship between energy stored as fat (internal stores) and energy stored as food (external stores). Internal stores were lowest in the winter, which corresponded to the highest levels of external stores. Internal stores were highest in the spring when external stores had been depleted, a strategy that would provide a large amount of internal energy available for reproduction in the spring. Plasma lipid levels also showed that lipids were being conserved during the winter.

Two-stage TOBEC models were accurate at predicting lean mass, however they performed poorly at predicting lipid mass. Direct models, in which lipid mass was predicted directly from body mass and TOBEC, predicted lipid mass more accurately in eastern woodrats than did two-stage models. Eastern woodrats showed definite seasonal changes in hematology, body composition, and food caches and TOBEC showed potential for estimating lipid mass of eastern woodrats.

SEASONAL VARIATION IN HEMATOLOGY, BODY COMPOSITION, AND FOOD CACHES OF EASTERN WOODRATS (NEOTOMA FLORIDANA)

A Thesis

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PREFACE

My thesis is organized into five chapters. Chapter one is an overall introduction to the thesis and the final chapter summarizes the conclusions of the thesis. All chapters are written in the style of the Journal of Mammalogy except chapter three, which is written in the style of Ecology.

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CHAPTER ONE
INTRODUCTION TO THESIS

Winter is a critical time for any small mammal in a seasonal environment due to decreased food availability and increased energy demands for thermoregulation. Non-hibernating small mammals face the challenge of acquiring sufficient energy to remain active throughout the year and to meet the requirements of a high metabolic rate. Small mammals use various behavioral and physiological adjustments to survive the winter. One behavioral adjustment is to store cached food for use during times of food scarcity. Animals that store all of their food at a single site are called larder hoarders (Vander Wall 1990). Temporal variation in food availability is considered to be an important variable leading to the evolution of larder hoarding behavior (Smith and Reichman 1984). The ability to defend a food cache against potential cache robbers is thought to be another important criteria for the evolution of larder hoarding (Smith and Reichman 1984; Vander Wall 1990). Obtaining and maintaining a larder hoard requires a considerable investment of time and energy. However, an individual must endure periods of resource scarcity in good condition if it is to breed successfully when conditions are favorable (Vander Wall 1990), thus increasing reproductive output or longevity.

A small mammal might make several physiological adjustments related to seasonal changes. Small mammals have been shown to increase oxygen carrying capacity in the winter, when metabolic activity was highest (Sealander 1962, 1964). Sealander (1962, 1964) showed that species of Peromyscus had the highest values of hematocrit (Hct), hemoglobin (Hb), and red blood cell (RBC) count in the winter and the same variables were lowest in the summer. Such hematological adjustments to seasonal change are primarily in response to seasonal differences in required oxygen capacity, however a small mammal can exhibit other physiological adjustments directly related to energy and nutritional demands of a season. During the winter, energy might be stored as body fat or as a food cache. The deposition of large quantities of fat is an integral part of the annual cycle of many hibernators (Morton and Tung 1971). However, how a larder hoarder that remains active throughout the year allocates resources to fat reserves or food reserves is not well understood (Vander Wall 1990). Storing energy as body fat for an animal that remains active during the period of cache use may be an inefficient strategy due to the loss of energy that would occur during the conversion of energy to fat and back again (Smith and Reichman 1984), but energy stored as fat is protected from loss to cache robbers or nutrient deterioration that may occur over time (Vander Wall 1990). However, for animals that rely on remaining active and mobile additional fat stores may be costly to carry around.

The eastern woodrat (Neotoma floridana) is a medium-sized rodent that remains active throughout the year, and relies on a larder hoard in the winter. Eastern woodrats begin caching food in September or October and continue until March (Rainey 1956). Post et al. (1993) found that the number of days of food stored in the caches of eastern woodrats ranged from 25 to 271. In addition, larger eastern woodrats stored more energy per gram of body mass in caches and more energy per total body mass than smaller individuals. Post et al. (1993) suggested that because smaller eastern woodrats do not appear to have sufficient energy stored in caches to last the winter they must spend a greater amount of time out of the house foraging for additional food. Post (1992) showed that some cached foods of eastern woodrats declined in nutritional value during storage. The loss of energy value in a food cache over winter would place a strain on eastern woodrats. However, eastern woodrats might manage their food cache in relation to the perishability of the food item by consuming more and caching less of a perishable item (Reichman 1988). In addition to loss of food to nutritional deterioration, Horne (1996) showed that white-footed mice (Peromyscus leucopus) are potential cache robbers of eastern woodrat caches.

The food cache is stored in an upper chamber of the eastern woodrat house where it can remain fresh for a considerable length of time and be protected from other animals (Rainey 1956). The eastern woodrat builds a large house, typically out of sticks, which provides escape from predators and protection from temperature extremes and moisture (Wiley 1980). A typical house is cone shaped, the degree to which depends on the availability of vertical support. Desert woodrats (Neotoma lepida) can build a complete house in 7-10 nights and have been demonstrated to add as many as 359 items to the house in one night (Bonaccorso and Brown 1972). Availability of cover is the most important habitat feature that determines shelter site selection (Rainey 1956). In the white-throated woodrat (Neotoma albigula), juvenile woodrats are looking for a house or a site to construct a house from mid-February through mid-October (Olsen 1973). Conrad (1995) found that for all insulation measurement trials taken from June to September in east-central Kansas, the temperatures inside the eastern woodrat house were cooler than ambient temperature. The mean insulation for occupied eastern woodrat houses was 3.5°C below ambient. Wooster (1990) found that temperatures in the nest region of the house were warmer when ambient temperature fell below 5°C in the winter. The breeding season of the eastern woodrat lasts from February to October in Kansas (Rainey 1956).

Little is known about seasonal changes in body fat in eastern woodrats and how they may be related to energy stored as food in the cache. Traditional methods of determining body fat have involved chemical extraction from a whole-body homogenate. Total body electrical conductivity (TOBEC) has become a popular non-invasive method of predicting fat-free mass in animals since its potential for use in ecological studies was first demonstrated by Walsberg (1988). The method has been used in several studies with birds (see literature cited in chapter four) and to a lesser extent with wild species of mammals (Bachman 1994, Voltura and Wunder 1996). The placement of an animal within a stable electromagnetic field results in a change in energy absorption, which can be converted into an index of conductance. Lean tissue is approximately 20 times more conductive than lipid due to the presence of sodium, potassium, and water in lipids. Therefore, the conductive index obtained by TOBEC is highly correlated with lean mass. Calibration of TOBEC with chemical extraction of lipids allows the prediction of fat-free mass from the conductive index. Many studies using TOBEC have reported high values of coefficient of determination, R^2. However, Morton et al. (1991) cautioned that high R^2 values in TOBEC studies might be misleading because of high relative error found between predicted and observed values of lipid mass, which was generally the variable of interest. Various regression models have been used to adjust for high relative error, such as including morphological measures into a multiple linear regression procedure. Voltura and Wunder (1996) cautioned against the use of TOBEC to study animals that remain relatively lean throughout the year, such as voles (Microtus spp.). Eastern woodrats may be an appropriate small mammal for use with TOBEC because they possess a greater lipid mass than voles.

The insulative properties of the eastern woodrat house have the potential to buffer the resident from the need for hematological adjustments to seasonal change that are found in other small mammals that do not inhabit such a highly modified microenvironment. The food cache limits the need to forage during extreme winter cold and, in turn, serves to further limit exposure to cold. Also, the role of energy stored as fat is not understood in a larder hoarding species such as the eastern woodrat. Therefore, the objectives of my research were 1) to determine if eastern woodrats exhibit seasonal variation in hematological values that are similar to those exhibited by other small mammals, 2) to determine if there is seasonal variation in body composition, serum chemistry, and energy stored as food in eastern woodrats, 3) to test for relationships between energy stored as fat and energy stored as food in the eastern woodrat in order to determine the seasonal strategy that larder hoarders may use in regards to those two variables, and 4) to evaluate the use of TOBEC to estimate lean and lipid mass in wild caught eastern woodrats.

Chapter two of my thesis details my research into the seasonal variation of hematological patterns of eastern woodrats in east-central Kansas. These patterns were expected to be primarily related to metabolic activity and seasonal demands for oxygen capacity. Chapter three details the nutritional aspects of my research that focus on energy stored as fat and food. Specifically, chapter three focuses on seasonal change in body composition, serum chemistry, and food caches of eastern woodrats of east-central Kansas. Chapter four details the evaluation of TOBEC as an estimator of lean and lipid mass in eastern woodrats. Conclusions of all chapters are summarized in chapter five.

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CHAPTER 2

SEASONAL CHANGES IN HEMATOLOGICAL VALUES OF EASTERN WOODRATS (NEOTOMA FLORIDANA)

ABSTRACT: Seasonal changes in hematological values have been documented in several species of small mammals. Changes in hematology are usually considered to reflect changes in metabolic activity related to the ambient temperatures of the season, with high values of packed cell volume (PCV), hemoglobin (Hb), and red blood cell (RBC) counts in the winter and low values in the summer. The eastern woodrat (Neotoma floridana) obtains the majority of its winter energy supply from a food cache stored in a house. The eastern woodrat house protects the resident from extreme temperatures and therefore may serve as a buffer to eliminate the need for seasonal hematological adjustments to ambient temperature. Eastern woodrats were collected in the fall and winter of 1999 and spring and summer of 2000 at three locations in east-central Kansas to obtain blood samples. Sex, age, and body mass did not significantly influence the hematological values measured, however season had a strong effect on all values, except mean cell volume (MCV). PCV and RBC counts were highest in the winter, lowest in the summer, and intermediate in fall and spring. Hb was lowest in the winter and highest in the spring and was paralleled by mean cellular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). PCV and Hb were positively correlated with body mass. Results indicated that high values of winter PCV and RBC count reflect the higher metabolic activity in that season, due to the increased energy demands of thermoregulation relative to summer. Low winter Hb levels were consistent with an iron-deficiency anemia and may be caused by a nutritional stress resulting from reliance on a food cache.

Key words: anemia, eastern woodrat, food cache, hematology, iron-deficiency, Neotoma floridana, nutrition.

INTRODUCTION

Seasonal changes in hematological values have been observed in many mammals as an adjustment to ambient temperature. Winter is a particularly critical season due to the increased energy demands for thermoregulation. Mass-specific metabolic rate is inversely correlated with body mass (Kleiber 1947), which places the highest energy demands per gram of body mass on small mammals. In addition, non-hibernating small mammals must acquire the additional energy necessary to remain active throughout the winter. Therefore, non-hibernating small mammals living in the cold might undergo various physiological and behavioral changes in relation to seasonal climatic change in order to survive the thermal challenges of winter (Sealander 1966).

General changes in metabolism, induced by temperature or other factors, influence the composition of blood, consequently hematological values might reflect seasonal fluctuations (Sealander 1964). Seasonal variation in hematological values have been studied in several non-hibernating small mammals including mice (Peromyscus spp.) and cotton rats (Sigmodon hispidus—Sealander 1962, 1964), bank voles (Clethrionomys glareolus—Newson 1962), northern red-backed mice (C. rutilus—Sealander 1966), meadow voles (Microtus pennsylvanicus—Mihok and Schwartz 1989), the Andean mouse (Akodon puer—Rosenmann and Ruiz 1993) the Djungarian hamster (Phodopus sungorus—Puchalski and Heldmaier 1986), chinchilla (Chinchilla lanigera—Jakubow et al. 1984), mountain possum (Trichosurus caninus) and brush-tailed possum (T. vulpecula—Barnett et al. 1979), and muskrat (Ondatra zibethicus—MacArthur 1984). Results of these studies have shown variation in the pattern and magnitude of the effects of season on hematological values. Sealander (1966) suggested that such differences could be explained by the effect of variation in the ambient temperature of the species' microenvironment on required metabolic activity.

Acclimation to cold would most obviously be accomplished by increasing levels of hematocrit (Hct), hemoglobin (Hb), or red blood cell (RBC) counts in order to increase oxygen carrying capacity (Rosenmann and Ruiz 1993). In deer mice and cotton rats, the highest values for Hct, Hb, and RBC counts occurred in the winter and the lowest values in the summer (Sealander 1962, 1964). The same pattern was found for muskrat in Manitoba (MacArthur 1983). Chinchilla showed a similar pattern except that the highest Hct values occurred in the summer (Jakubow et al. 1984). Sealander (1962) suggested that high winter values of Hct, Hb, and RBC count reflected higher metabolic activity and that elevated heat production via higher metabolic rate was a prominent feature of cold acclimation in small mammals. However, northern red-backed mice in interior Alaska do not show any significant seasonal change in Hct or Hb levels, possibly due to the advantage of living in a much warmer winter microenvironment (Sealander 1966). The Djungarian hamster showed no seasonal change in Hb level, but showed higher RBC counts and a slight increase in Hct during winter (Puchalski and Heldmaier 1986). The Andean mouse showed no significant change in Hct over seasons, but winter was characterized by an increase in RBC counts and a decrease in mean cell volume (MCV), which possibly allowed for an enlarged diffusion area per unit volume of blood (Rosenmann and Ruiz 1993). Newson (1962) found high Hb levels in the first winter of study on bank voles, however the second winter showed no clear pattern with variable Hb values. Meadow voles showed a significant decrease in Hct and Hb levels at the onset of winter, which suggested anemia (Mihok and Schwartz 1989). Barnett et al. (1979) found that Hb levels in the mountain possum and brush-tailed possum were low in the first winter of study, high in the second, and RBC counts of the mountain possum were highest in winter. In addition to seasonal changes in ambient temperature, other factors that could influence hematological values include age, reproductive condition, sex, and nutritional state.

The eastern woodrat (Neotoma floridana) is a medium-sized rodent that remains active throughout the year (Wiley 1980). In Kansas, the eastern woodrat survives winter due to the protective nature of its house and a stored food cache (Rainey 1956). The eastern woodrat house is typically cone-shaped and almost exclusively built with sticks, however objects such as rocks, dung, bark, broken glass, and bones are also commonly found in houses (Rainey 1956). The insulative properties of the house allow for a warmer microenvironment in the winter, a cooler microenvironment in the summer, and protection from extreme temperatures. Wooster (1990) found that temperatures in the nest region of the house were warmer when ambient temperature fell below 5°C and Conrad (1995) found that temperatures inside the house were cooler than ambient in the summer. The food cache of the eastern woodrat serves as the primary energy source during the winter. Eastern woodrats have been shown to store from 25 to 271 days of energy in food caches (Post et al. 1993). Such a large store of energy available during the winter could substantially limit the need to forage outside of the house and in turn limit exposure to extreme cold temperatures.

The eastern woodrat house represents a highly modified microenvironment with the potential to buffer the resident from the effects of seasonal temperature fluctuations and the benefit of a food cache limits the need to forage outside of the house. As a result, seasonal variations in hematological values of the eastern woodrat might not be as evident as it is in other species of small mammals that have been studied. The objective of my study was to determine if there was seasonal variation in selected hematological values of eastern woodrats in east-central Kansas.

MATERIALS AND METHODS

Study Area.—Eastern woodrats were collected from three study areas within Chase, Coffey, and Lyon counties, Kansas. The first area, the Ross Natural History Reservation (RNHR—38° 30' N, 96° 20' W) an area owned by Emporia State University, is an 80 ha area located on the eastern edge of the Flint Hills region of east-central Kansas and consists of grassland, edge habitats, woody areas, and riparian zones. Eastern woodrat houses are found predominantly in the hedgerow areas of RNHR, which are dominated by Osage orange (Maclura pomifera) and red cedar (Juniperus virginiana). Other abundant tree and shrub species on RNHR include honey locust (Gleditsia triacanthos), cottonwood (Populus deltoides), American elm (Ulmus americana), hackberry (Celtis occidentalis), green ash (Fraxinus pennsylvanica), rough-leaved dogwood (Cornus drummondii), fragrant sumac (Rhus aromatica), wild plum (Prunus americana), Missouri gooseberry (Ribes missouriense), and buckbrush (Symphoricarpos orbiculatus—Conrad, 1995). The second study area was a wooded riparian area located on private land (38° 25' N, 95° 57' W) in Coffey County, Kansas. Dominant tree and shrub species at the area were similar to those at RNHR. The third study area was located on the Flint Hills National Wildlife Refuge (FHNWR—38° 19' N, 95° 56' W), which consists of 7,493 ha containing large wooded areas and numerous riparian zones. The trapping site was located on a wooded south-facing ridge. Dominant tree and shrub species were similar to those found at RNHR, but also included black walnut (Juglans nigra) and oak (Quercus spp.).

Trapping.—Eastern woodrats were live trapped during the fall (20-22 October 1999), winter (15-17 December 1999), spring (11-14 March 2000), and summer (8-22

August 2000). Ten eastern woodrats (six males and four females) were trapped in the fall and seven (two males and five females) were trapped in the summer from RNHR. Seven eastern woodrats (four males and three females) were trapped in the winter from the private property and 11 (two males and nine females) were trapped in the spring at FHNWR. Eastern woodrat houses were located at each study site and two Tomahawk live traps (sizes varied) were set at each house. Traps were set in the evening and checked in the morning, times varied by season of trapping. Each trap was baited with a mixture of peanut butter and oatmeal and supplied with fiberfill in the winter and spring trapping seasons. Trapped eastern woodrats were taken back to the laboratory at Emporia State University where body mass and sex were determined.

Hematological Analysis.—I anesthetized eastern woodrats with 0.22 to 0.26 ml (depending upon body size) of ketamine (Fort Dodge Laboratories, Fort Dodge, Iowa). Approximately 1-3 ml of blood were drawn by heart puncture from each eastern woodrat into a heparinized syringe. Once the blood was collected, each eastern woodrat was killed by cervical dislocation following the guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998). Packed cell volume (PCV) was determined by the microhematocrit method. Hb was measured by the cyanomethemoglobin method (Sigma Chemicals, St. Louis, Missouri). RBC counts were determined by diluting the blood in a standard red blood cell pipette (1:200) with a 0.9% NaCl solution. The diluted blood was placed on a hemocytometer and cells were counted. MCV, mean corpuscular hemoglobin concentration (MCHC), and mean cellular hemoglobin (MCH) were determined from the values for PCV, Hb, and RBC count. MCV was calculated as PCV divided by RBC count and then multiplied by 10^6. MCHC was calculated as Hb divided by PCV and multiplied by 100. MCH was calculated as Hb divided by RBC count and then multiplied by 10^9.

Statistical Analysis.—All statistical analyses were performed by using the Statistical Analysis System (SAS Institute, Inc. 1999). Differences in sex and season for each blood parameter and body mass were tested by using a two-way analysis of variance (ANOVA) with the general linear model procedure (PROC GLM—SAS Institute, Inc. 1999) to account for unequal sample sizes. RBC counts were log transformed to achieve normality. Correlations of body mass with hematological values and among hematological values were tested by using Pearson's Correlation (PROC CORR—SAS Institute, Inc. 1999). Four eastern woodrats (one fall and three summer) were infected with botfly (Cuterebra sp.) larvae, however they were included in analysis because omitting the fall individual did not change significance of any tests and the three individuals from the summer did not have values differing significantly from other summer individuals.

RESULTS

All hematological values of eastern woodrats, except MCV, showed seasonal variation (Table 1). RBC count (*F* = 6.55, *d.f.* = 1, 27, *P* = 0.02) was the only variable to show a significant difference for sex. However, when RBC count comparisons for sex were made within season the only significant difference was for spring males (*n* = 2) and spring females (*n* = 9, *t* = 2.54, *d.f.* = 27, *P* = 0.02), therefore data for males and females were combined. There was not a significant difference in body mass between males and females (*F* = 1.31, *d.f.* = 1, 27, *P* = 0.26), however male body mass was typically higher than females. The only seasonal difference is body mass was that summer body mass was significantly lower than spring (*t* = 3.06, *d.f.* = 27, *P* = 0.005).

PCV values showed a significant difference between seasons (*F* = 12.90, *d.f.* = 3, 27, *P* < 0.0001) with highest values occurring in winter and the lowest in summer. PCV increased significantly from fall to winter (*t* = 3.73, *d.f.* = 27, *P* = 0.0009), from a mean value of 39.7 to 45.7%. There was no change in PCV from winter to spring (*t* = 0.20, *d.f.* = 27, *P* = 0.84), followed by a significant decrease from spring to summer (*t* = 12.05, *d.f.* = 27, *P* < 0.0001).

Hb concentrations varied significantly by season (*F* = 16.11, *d.f.* = 3, 27, *P* < 0.0001). There was a significant decrease in Hb from fall to winter (*t* = 2.33, *d.f.* = 27, *P* = 0.03), which represented a 15.2% reduction in mean Hb concentration. Spring showed a significantly higher Hb concentration than winter (*t* = 15.64, *d.f.* = 27, *P* < 0.0001) and Hb significantly decreased from spring to summer (*t* = 11.60, *d.f.* = 27, *P* < 0.0001). Highest Hb concentrations occurred in the spring and the lowest occurred in the winter.

Table 1. Seasonal changes in hematological values of eastern woodrats (Neotoma floridana). Values are presented as mean ± standard error.

Variable	Fall (n = 10)	Winter (n = 7)	Spring (n = 11)	Summer (n = 7)
Body mass (g)	250.0 ± 24.0	256.8 ± 14.8	280.4 ± 15.2	215.7 ± 15.4
PCV (%)	39.7 ± 1.2	45.7 ± 1.2	44.0 ± 1.2	35.4 ± 0.9
Hb (g/dl)	11.4 ± 0.5	9.4 ± 0.4	14.5 ± 0.6	10.7 ± 0.2
RBC Count ($10^6/mm^3$)	7.36 ± 0.31	8.84 ± 0.31	7.31 ± 0.41	6.11 ± 0.18
MCV (μm^3)	54.1 ± 2.11	52.0 ± 2.08	62.0 ± 3.79	57.9 ± 0.99
MCH (pg)	15.5 ± 0.06	10.7 ± 0.06	20.4 ± 0.01	17.6 ± 0.03
MCHC (%)	28.8 ± 0.01	20.6 ± 0.4	33.0 ± 0.01	30.4 ± 0.01

Seasonal changes in RBC counts paralleled that of PCV with highest values occurring in the winter and lowest values in the summer. RBC counts showed a significant increase from fall to winter (*t* = 3.04, *d.f.* = 27, *P* = 0.005), no significant change from winter to spring (*t* = 1.68, *d.f.* = 27, *P* = 0.11), and a significant decrease from spring to summer (*t* = 3.11, *d.f.* = 27, *P* = 0.004).

MCV showed no significant variation with sex (*F* = 1.19, *d.f.* = 1, 27, *P* = 0.28) or season (*F* = 0.87, *d.f.* = 3, 27, *P* = 0.47). Highest values were found in the spring and lowest in the winter. MCHC showed significant seasonal variation (*F* = 29.54, *d.f.* = 3, 27, *P* < 0.0001). Winter values of MCHC were significantly lower than fall values (*t* = 12.56, *d.f.* = 27, *P* < 0.0001) and spring values were significantly higher than winter values (*t* = 20.82, *d.f.* = 27, *P* < 0.0001). MCHC was not significantly different from spring to summer. Highest values of MCHC were in the spring and lowest in the winter.

MCH varied significantly by season (*F* = 10.62, *d.f.* = 3, 27, *P* < 0.0001). MCH decreased significantly from fall to winter (*t* = 3.00, *d.f.* = 27, *P* = 0.006) and showed a pronounced increase from winter to spring (*t* = 12.27, *d.f.* = 27, *P* < 0.0001). There was no difference in MCH from spring to summer (*t* = 1.12, *d.f.* = 27, *P* = 0.27). The highest MCH values occurred in spring and the lowest in winter.

Body mass was positively correlated with PCV (*r* = 0.35, *d.f.* = 33, *P* = 0.038) and Hb (*r* = 0.36, *d.f.* = 33, *P* = 0.03), however RBC counts showed no correlation with body mass (*r* = 0.24, *d.f.* = 33, *P* = 0.16). In addition, body mass was not correlated with MCV (*r* = -0.03, *d.f.* = 33, *P* = 0.84). Despite the drop in winter Hb concentrations, PCV and Hb were positively correlated (*r* = 0.43, *d.f.* = 33, *P* = 0.011). PCV was also positively correlated with RBC counts (*r* = 0.49, *d.f.* = 33, *P* = 0.003). RBC counts were inversely correlated with MCV (*r* = -0.70, *d.f.* = 33, *P* < 0.0001), MCHC (*r* = -0.38, *d.f.* = 33, *P* = 0.025), and MCH (*r* = -0.64, *d.f.* = 33, *P* < 0.0001).

DISCUSSION

Seasonal changes in hematological values can reflect changes in metabolic activity due to different ambient or microenvironmental temperatures. The hematological values of eastern woodrats, with the exception of MCV, showed seasonal fluctuations. The values for PCV, Hb, and MCHC found in my study fell within the range of values reported by Sealander (1964) for five adult eastern woodrats in northwest Arkansas.

Sex was not a factor influencing the hematology of eastern woodrats in my study. Only RBC counts showed a significant effect of sex, however this was likely due to a disproportionately high number of females included in the spring sample. Studies have varied in demonstrating an effect of sex on hematology in small mammals. Sealander (1964) found no significant differences between sexes in Hb, Hct, or RBC diameter when pregnant and lactating females were excluded. None of the female eastern woodrats in my study were pregnant, however there were females observed to be lactating in the spring sample. Sexual dimorphism in Hct and Hb was reported for the mountain possum and brush-tailed possum, however the brush-tailed possum also showed a sexual dimorphism in body mass (Barnett et al. 1979). Newson (1962) found consistent differences in values for male and female bank voles and attributed those effects to breeding. However, the evidence was strong that breeding was not acting alone to influence the values. Contrary to those results, Goldfinger and Goldfinger (1964) found close average values for male and female bank voles and common shrews (Sorex araneus). Additionally, MacArthur (1984) found no differences in hematological values between male and female muskrats.

Age and body mass can have an effect on hematological values. Juveniles have been shown to have a larger MCV and lower RBC count than adults (Sealander 1964) and Withers et al. (1979) found a good correlation between RBC counts and body mass in Arctic small mammals. Although hematological values for juvenile small mammals were different from adults, values for subadults were not significantly different from adults (Sealander 1964). In my study, all mean values of seasonal body mass fell within the range typical of adult eastern woodrats (174-384g—Rainey 1956) and all eastern woodrats included in my study were adults. The only season to show a significant effect for body mass was summer, which had a lower mean body mass than the fall, winter, or spring eastern woodrats. Body mass can be indicative of the age of an individual, in that body mass is typically higher for older individuals. The lack of significant changes in MCV across seasons in my study indicated that age was not a factor because juveniles tend to have larger erythrocytes. In addition, MCV and RBC counts were not correlated with body mass, which indicated that the seasonal fluctuations observed in eastern woodrats were independent of the body mass of individuals sampled.

The most likely adjustment of a small mammal to meet the increased energy demands for heat production in the winter would be to increase oxygen transport by increasing PCV, Hb, or RBC counts (Rosenmann and Ruiz 1993; Sealander 1962). Cotton rats, deer mice, and fulvous harvest mice (Reithrodontomys fulvescens) demonstrated such an adjustment by distinctly showing the highest Hb and Hct values in the winter, when metabolic activity is highest, and lowest Hb and Hct values in the summer (Sealander 1962). The significant increase in winter PCV and RBC counts of eastern woodrats in my study was consistent with such a pattern. The high levels of PCV and RBC counts in the winter likely reflected higher metabolic activity relative to the summer. Accordingly, the lowest values for PCV and RBC counts in eastern woodrats were found during the summer. One major difference between expected patterns of hematological adjustments and what was observed in eastern woodrats was the drop in Hb levels in the winter. Although PCV and RBC counts increased from fall to winter, Hb declined to its lowest level. Sealander (1960) provided some evidence that Hct and Hb do not always parallel each other. The significant decrease in winter Hb in my study was accompanied by significant decreases in MCHC and MCH, but not MCV, which suggested that the decline in Hb was not due to smaller erythrocytes but to a lower total Hb concentration and, in turn, smaller amounts of Hb contained in each erythrocyte. The correlation between RBC counts and MCH also indicated that when RBC counts were highest, which was in the winter, values for MCH were lowest. Such a pattern suggests that low winter Hb levels were due to the occurrence of an iron-deficiency anemia in eastern woodrats. The production of Hb is dependent upon iron and oxygen carrying capacity is directly related to Hb concentration, therefore anemia would represent a significant stress to wintering eastern woodrats. However, it is possible that the eastern woodrats in my study compensated for low Hb levels by increasing the oxygen affinity of the Hb, which I did not measure.

Anemia is not an undocumented occurrence in small mammals studied in the wild. Mihok and Schwartz (1989) documented a normocytic and normochromic anemia at the onset of winter in meadow voles. Mean Hct in their study was 51% and individuals with a Hct under 42% were considered anemic. The largest number of anemic meadow voles was captured at the onset of winter and anemia was most common at temperatures below 5°C. Anemia was not related to prevalence of blood parasites and was not genetic because of the random occurrence in the population. The anemia found in the meadow vole population was characterized by low numbers of erythrocytes, however iron stores were abundant. The authors suggested that nutritional stress was the most likely candidate leading to anemia, which was evidenced by an annual minimum in protein and energy reserves that would reflect a change in the availability of a critical nutritional resource in winter. Despite the evidence to suggest nutritional stress as the cause of the anemia, the authors concluded, based on dissections, that the anemic conditions observed in the meadow voles were due to a sudden, large loss of blood, which likely occurred through erosions of the glandular epithelium of the stomach.

Although the anemia documented by Mihok and Schwartz (1989) was significant, the evidence in eastern woodrats pointed towards an anemia related to low Hb levels, not due to low numbers of erythrocytes as found in meadow voles. Barnett et al. (1979) did find low Hb levels in the first winter of study with mountain possums and brush-tailed possums that were not associated with a lowered Hct. In the second winter of their study, Hb levels were at their peak. The condition present in the two species in the first winter was consistent with an iron-deficiency anemia like that found in eastern woodrats. If Hb was being influenced by nutritional state, then Hb levels would likely vary depending on food availability by season and year. Therefore, not surprisingly, Hb levels in the mountain and brush-tailed possums returned to high levels in the second winter. Hellgren et al. (1993) found that Hb and RBC counts in black bears (Ursus americanus) could be used to discriminate between good and poor years of mast and berry production and that Hb was higher during all active periods of good years compared to poor years, although levels during hibernation were similar. The year in which eastern woodrats were trapped for my study could be characterized as a poor year in terms of food supply. The summer of 1999 was a drought year, which was followed by a mild winter from December to February, 1999-2000. Francis and Strong (1938) found that Hb levels of laboratory rats maintained on a higher quality fox chow diet were higher than those fed oatmeal chow. They suggested that diet was probably the most important extrinsic factor capable of changing the amount of Hb in the blood of animals. Goldfinger and Goldfinger (1964) also found low winter Hb levels in bank voles and common shrews and agreed with the importance of diet. They stated that Hb levels directly reflected changes in diet, while RBC counts were influenced primarily by rate of metabolism.

The food cache of an eastern woodrat, although beneficial for winter survival, could play a role in causing a nutritional stress that may lead to anemia. Post et al. (1993) found that eastern woodrats stored from 25 to 271 days of energy in the food cache. They also found that larger eastern woodrats had more days of energy stored than smaller eastern woodrats. Smaller eastern woodrats that did not appear to have sufficient energy stored to last throughout the winter would need to spend a greater amount of time outside of the house foraging for additional food. If smaller eastern woodrats were encountering a greater nutritional stress than larger individuals and Hb levels were influenced by such a stress than a relationship between Hb and body mass would be expected. In fact, Hb and body mass were positively correlated in my study, which suggested that such a relationship with nutritional state might exist. However, the relationship between Hb and body mass was not significant within the winter, which could be the result of a small sample size. In addition to the limited food availability that would come from being restricted to a food cache, the nutritional composition of the cached food might change over time. Post (1992) found that although dry mass of four commonly cached food types did not change over time, the nutritional composition did change over winter. The greatest reduction was in the lipid content of rough-leaved dogwood fruit pulp, which resulted in a decline in the energy content of the same food type. Another potential nutritional stress could result from the energetic costs of consuming plant secondary compounds (Mihok and Schwartz 1989; Thomas et al. 1988). Red cedar makes up a large portion of the winter diet of eastern woodrats (Post 1991) and red cedar foliage contains large quantities of phenolic compounds. Norris (2000) found that red cedar foliage contained 19.56% lignin, which is a phenolic compound.

Correlations between hematological values and body mass have not been well documented. Goldfinger and Goldfinger (1964) and Sealander (1962) did not find any consistent correlations between body mass and hematological measurements. Withers et al. (1979) did find good correlations between body mass and RBC counts and MCV in an interspecific comparison of several Arctic small mammals. The relationship of high RBC counts and low MCV was attributed to maintaining the surface:volume ratio of the erythrocyte. I found no significant correlations between RBC counts or MCV with body mass. However, PCV and Hb were correlated with body mass. The former could be attributed to a larger blood volume necessary to accommodate a greater tissue mass in larger individuals and the latter might reflect the same pattern or a disproportionate nutritional stress, as discussed previously.

Puchalski and Heldmaier (1986) found that in Djungarian hamsters RBC counts increased in winter and were paralled by a reduction in MCV. They suggested that decreased MCV with increased RBC counts might provide an enlarged diffusion area for blood gases per unit volume of blood. The reduction in MCV, along with a constant Hct, worked to prevent the disadvantages of increased blood viscosity. In addition to an enlarged diffusion area, acquisition of a small RBC size during the winter in the Andean mouse was suggested to favor a denser capillary network for a rapid cardiac cycle (Rosenmann and Ruiz 1993). The same correlation of increased RBC counts and decreased MCV was found in eastern woodrats. Although MCV did not significantly change over seasons, lowest mean MCV and highest mean RBC counts did occur in winter. Increasing the diffusion area per unit volume of blood might have the potential to offset low Hb levels by transporting oxygen more efficiently. However, because PCV increased in winter along with RBC counts, the advantage of a smaller MCV on blood viscosity might be counteracted.

Patterns of hematological changes in eastern woodrats were related to seasonal changes in ambient temperature, which reflected seasonal differences in metabolic activity. For PCV and RBC counts, highest values occurred in the winter when metabolism for thermoregulation should be highest and the lowest values occurred in the summer. Fall and spring represented intermediate levels of PCV and RBC counts. Although Hb levels were expected to be at their highest levels in the winter, in order to meet needs for increased oxygen capacity, they were at their lowest and showed evidence of an iron-deficiency anemia. Low Hb levels probably reflected the effects of a nutritional stress on wintering eastern woodrats. The low winter Hb levels shifted the peak levels into the spring, which coincided with the beginning of the breeding season. Although sex was not determined to be playing a major role in my study, it is likely a factor influencing hematological values during the breeding season. The thermal benefits of the eastern woodrat house were not enough of a barrier to temperature fluctuations to eliminate the need for physiological adjustments and eastern woodrats showed seasonal changes in hematological values similar to those found in other small mammal species. However, the effect of nutritional stress in food-caching species on Hb levels requires further study.

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CHAPTER THREE

SEASONAL VARIATION IN SERUM CHEMISTRY, BODY COMPOSITION, AND FOOD CACHES OF EASTERN WOODRATS (*NEOTOMA FLORIDANA*)

Abstract: Few studies have investigated the relationship between energy stored as food (external stores) and energy stored as fat (internal stores) in larder-hoarding animals. A larder hoarding animal invests a considerable amount of energy in collecting and defending its larder, or food cache. The benefits of the food cache should outweigh the costs of maintaining it in order for the hoarding behavior to persist. The eastern woodrat (*Neotoma floridana*) is a medium-sized rodent that remains active all year and stores a food cache for use during the winter. Eastern woodrats were trapped in the fall, winter, spring, and summer of 1999-2000 to study their serum chemistry, body composition, and food cache composition. Serum chemistry (plasma lipids and proteins) was determined for individuals from all seasons. Plasma lipid levels were highest in the fall and lowest in the winter, however plasma protein concentrations remained constant. Fall, winter, and spring eastern woodrats were also analyzed for body and food cache composition. Chemical extractions of lipid revealed that percent body fat was highest in the spring, but not different between the fall and winter. Energy content of food caches was highest in the winter, when internal energy stores were lowest, and at a minimum in the spring, when internal energy stores were highest. Eastern woodrats employ a strategy of maximizing external energy stores for use in the winter and conserving internal fat stores during that time. The potential pay-off of such a strategy would be a high amount of internal energy stores available for reproduction at the beginning of the breeding season in the spring.

Key words: body composition, eastern woodrat, food cache, larder hoarding, lipids, *Neotoma floridana*, percent body fat, serum chemistry.

INTRODUCTION

A larder hoarding animal invests a considerable amount of energy in obtaining and defending its food cache. For such a strategy to persist, the pay off to the larder hoarder must be greater than the costs of gathering and maintaining the larder. The evolution of food hoarding has been suggested to be a response to temporal food scarcity or nutritional deficit caused by the interaction of two variables: food availability and energy demand (Smith and Reichman 1984, Vander Wall 1990). One possible explanation for the evolution of larder hoarding behavior is the "security hypothesis", which states that an animal that carries its food to a secure refuge to feed would benefit from a decreased risk of predation (Bindra 1948). If such a behavior was selected, it could lead to larder hoarding. One aspect of larder hoarders that has not been well studied is the allocation of resources to either fat reserves or food reserves (Vander Wall 1990). Lipids represent the primary mode of energy storage in animals and the deposition of large quantities of fat is an integral part of the annual cycle of many hibernators (Morton and Tung 1971). Smith and Reichman (1984) suggested that storing energy as body fat would be an unsuitable strategy for an animal that remains active throughout the period of cache use because it would reduce mobility and be physiologically inefficient due to the loss of energy that would occur during the conversion of energy to fat and back again. However, Vander Wall (1990) stated that even for a food hoarder the storing of energy as body fat would have the advantages of preventing loss of food stores to deterioration over time and/or loss from cache robbers.

The eastern woodrat (*Neotoma floridana*) is a larder hoarder that remains active throughout the year, and relies on its food cache to survive the reduction in food availability during the winter (Wiley 1980). Eastern woodrats begin caching food in September or October and continue until March (Rainey 1956). Post et al. (1993) found that the number of days of food stored in the caches of eastern woodrats ranged from 25 to 271 and that caches in October had a mean energy content of 3,682 ± 53 kcal while December caches contained 2,369 ± 22 kcal. In addition, larger eastern woodrats stored more energy per gram of body mass in caches and more energy per total body mass than smaller individuals. They suggested that because smaller eastern woodrats did not appear to have sufficient energy stored in caches to last the winter they must spend a greater amount of time out of the house foraging for additional food. Post (1992) showed that some cached foods of eastern woodrats decline in nutritional value during storage. The loss of energy value in a food cache over winter would place an additional strain on eastern woodrats. However, eastern woodrats might manage their food cache in relation to the perishability of the food item by consuming more and caching less of a perishable item (Reichman 1988). Horne (1996) has shown that white-footed mice (*Peromyscus leucopus*) are potential cache robbers of eastern woodrat food caches. Losses of energy to cache robbers and nutrient deterioration might have the potential to cause eastern woodrats to store energy as body fat.

The strategy of storing energy as lipids also varies between species that hibernate and those that go into torpor. Jameson and Mead (1964) found that golden-mantled ground squirrels (*Spermophilus lateralis*) showed a threefold increase in the lipid index in September, just prior to dormancy. In the same study, Jameson and Mead (1964) found that lodgepole chipmunk (*Tamias speciosus*) and yellow-pine chipmunk (*Tamias amoenus*) showed an increase in lipid index prior to dormancy, however it was not accompanied by an increase in body mass as with the golden-mantled ground squirrel. The authors suggested the lower increase in lipid index and mass in chipmunks was due to food stores in the den that were used periodically during dormancy. Forbes (1966) found little evidence of fat accumulation in eastern chipmunk (*Tamias striatus*) and least chipmunk (*Tamias minimus*) and suggested that they rely primarily on hoarded food as the winter energy supply. Blake (1972) also showed that golden-mantled ground squirrels have a period of fattening as part of the hibernal cycle and reach peak fat storage just prior to hibernation. The prairie vole (*Microtus ochrogaster*) does not hibernate, but has been shown to increase capacity for non-shivering thermogenesis by 136% in the winter (Wunder 1985). In addition, *Microtus* spp. lost mass in the winter or changed growth rates. Seasonal changes in body fat levels corresponding to food cache use in eastern woodrats have not been studied.

Hematology and serum chemistry can be indicators of an animal's nutritional status (Seal and Hoskinson 1978). Several studies have used serum chemistry to assess physiological and nutritional state of mammals, particularly ungulates (Seal et al. 1972, Seal and Hoskinson 1978, Warren et al. 1981). Serum chemistry has also been used to discriminate between habitat quality in black bears (*Ursus americanus*, Hellgren et al. 1993) and to study the effects of food deprivation in badgers (*Taxidea taxus*, Harlow and Seal 1981). There has been limited use of serum chemistry to assess nutritional condition of small mammals. Studies of seasonal variation in small mammals have focused on hematological values, such as packed cell volume (PCV), hemoglobin (Hb), and red blood cell (RBC) count. Metabolic energy needs can only be met by the catabolism of fat in adipose tissue or protein in lean body mass or some combination of the two (Runcie and Hilditch 1974). Therefore, measures of plasma lipids (triglycerides and cholesterol) and plasma protein concentrations would be useful in assessing the strategy of a larder hoarder for storing energy as body fat or food stores and when and to what extent fat stores are mobilized. The objectives of my study were: 1) to determine if there were seasonal changes in body composition, serum chemistry, and food caches of eastern woodrats and 2) to determine relationships between energy stored as body fat and energy stored as food in eastern woodrats.

MATERIALS AND METHODS

Study Area

Eastern woodrats were studied at three sites in east-central Kansas, located within Chase, Coffey, or Lyon counties. See chapter two for descriptions of each study site.

Trapping

Eastern woodrats were trapped during the fall (20-22 October 1999), winter (15-17 December 1999), spring (11-14 March 2000), and summer (8-22 August 2000). Ten eastern woodrats (six males and four females) were trapped in the fall, seven (four males and three females) in the winter, 11 (two males and nine females) in the spring, and seven (two males and five females) in the summer. Eastern woodrat houses were located at each study site and two Tomahawk live traps (sizes varied) were set at each house. Traps were set in the evening and checked at sunrise. Each trap was baited with a mixture of peanut butter and oatmeal and supplied with fiberfill in the winter and spring trapping periods. The eastern woodrat that was trapped at a house was assumed to be the resident of the house. Each house at which an eastern woodrat was trapped was given a unique identification number that corresponded to the house resident. Trapped eastern woodrats were taken back to the laboratory at Emporia State University where body mass and sex were determined. Eastern woodrats trapped in the summer were not included in the body composition and food cache portions of the study.

Serum Chemistry Analysis

I anesthetized eastern woodrats with 0.22 to 0.26 ml of ketamine (Fort Dodge Laboratories, Fort Dodge, Iowa). Approximately 1-3 ml of blood were drawn into a heparinized syringe by heart puncture from each eastern woodrat. Once the blood was collected, each eastern woodrat was killed by cervical dislocation following the guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998), placed in a plastic bag, and frozen. Routine hematological analyses were performed and those results were reported in chapter two. Plasma was separated by centrifugation with a Fisher Scientific Micro14 Centrifuge at 5,000 RPM for four minutes. Plasma protein concentration was determined by the total protein method (Sigma Chemicals, St. Louis, Missouri). Serum cholesterol levels were measured by the direct determination method following Zlatkis et al. (1953). Triglyceride concentrations were determined with Sigma Chemicals Kit 336-10 (St. Louis, Missouri).

Body Composition by Chemical Extraction

Carcasses were thawed and dried at 80°C in a drying oven (Kerr et al. 1982) to constant mass. Dry mass of the carcass was subtracted from total body mass of the individual at time of trapping to obtain water content. Each carcass was ground in a coffee grinder and homogenized carcasses were sent to the Department of Zoology, University of Western Ontario, London, Ontario, Canada for chemical extraction. Cellulose thimbles were dried to constant mass (thimble) and then filled with approximately 10 or 40 g, depending on thimble size, of the dry, ground carcass. Thimbles and samples were dried to constant mass (thimble + sample dry mass) and then extracted with petroleum ether (Dobush et al. 1985) in a modified Soxhlet apparatus. Following extraction of lipids, thimbles and lean samples were returned to the ovens and dried to constant mass (thimble + lean sample dry mass). Fat content was calculated as :

$$\text{Total carcass fat} = \frac{\text{sample fat}}{\text{sample dry mass}} \times \text{total carcass dry mass}$$

or

$$\frac{(\text{thimble} + \text{sample mass})-(\text{thimble} + \text{lean sample dry mass})}{(\text{thimble} + \text{sample dry mass})-(\text{thimble})} \times \text{total carcass dry mass.}$$

Chemical extractions were performed on the entire dry sample of the carcass. Petroleum ether is preferred for studies of body composition because it extracts lipids completely, but does not extract nonlipid material (Dobush et al. 1985). Fat-free mass was calculated as the total body mass of the individual (including water) minus the lipid mass determined by extraction. Percent body fat was calculated as the lipid mass determined from extraction divided by total body mass multiplied by 100. Percent body water was calculated as water content determined from drying divided by total body mass and multiplied by 100.

Food Cache Analysis

Within two weeks of trapping, food caches were retrieved from houses where eastern woodrats had been trapped. Food caches were not recovered from summer houses because there is no cached material during that time. Before removing the food cache, external height and diameter of the house were measured. Volume of the house was calculated by using the formula for a cone. Food caches were not recovered from three houses in the fall, one in winter, and two in the spring. These houses were considered auxiliary houses and were not included in statistical analyses. An effort was made to recover as much of the cache as possible, however some of the dried leaf material fragmented with handling and was not recovered. The amount of unrecoverable leaf fragment or cache material from each cache was estimated to be less than 5% of the total cache.

Caches were sorted and plant types identified to species. Total dry mass of each food type was determined as well as the total dry mass of the entire cache. The energy content of the edible portion of the cache was determined as the product of the dry mass of each food type and its energy content (kcal/g). For energy values of each portion of the cache, I used values reported in the literature or determined by bomb calorimetry (Table 1). A single energy value was used for all dicot leaf material. Eastern woodrats consume only the foliage of red cedar (*Juniperus virginiana*, Post 1991), therefore the energy of red cedar fruits or stems were not included in energy calculations. Also, inedible portions of food types such as honey locust (*Gleditsia triacanthos*) seed pods and black walnut (*Juglans nigra*) shells were not included in energy calculations. In addition, eastern woodrats do not consume the pulp of Osage orange (*Maclura pomifera*), hence only the seeds were included as sources of energy. Total energy content of the cache was determined as the sum of the total energy content of all food types found in the cache.

Body mass of the cache owner was used to estimate average daily metabolic rate (ADMR = $2.297W^{-0.5}$, French et al. 1976) of each eastern woodrat in kcal g^{-1} day^{-1}. Average daily energy requirement (ADER, kJ/day) was calculated as the product of ADMR and body mass. The number of days of energy stored in each food cache was determined by dividing the total energy content of the food cache by the average daily energy requirement (kJ/day) of the cache owner.

Statistical Analysis

Differences between sex and season for protein concentration, serum cholesterol level, and triglyceride concentration were tested by using a two-way analysis of variance

Table 1. Energy values of food types found in eastern woodrat (*Neotoma floridana*) food caches in east-central Kansas 1999-2000.

Food Type	Energy value (kcal/g)	Reference
Dicot leaf material	4.2	Golley 1961
Grass	3.9	Golley 1961
Kentucky coffee-tree (*Gymnocladus dioica*) seeds	5.1	Golley 1961
Honey locust (*Gleditsia triacanthos*) seeds	4.0	Horne 1996
Stick cambium	4.0	Horne 1996
Osage orange (*Maclura pomifera*) seeds	6.0	Horne 1996
Red cedar (*Juniperus virginiana*) foliage	4.6	Post 1991
Rough-leafed dogwood (*Cornus drummondii*) fruit	6.0	Post 1991
Coralberry (*Symphoricarpos orbiculatus*)	4.6	Post 1991
Black walnut (*Juglans nigra*)	6.6	Souci et al. 1994
Fungi	3.9	bomb calorimetry
Eastern prickly pear (*Opuntia humifusa*)	3.2	bomb calorimetry

(ANOVA) with a general linear model procedure (PROC GLM, SAS Institute, Inc. 1999) to account for unequal sample sizes. Data from fall, winter, spring, and summer were included in the test. Differences between sex and season for percent body fat, percent body water, and house volume of all eastern woodrats trapped in the fall, winter, and spring were tested with a two-way ANOVA by using PROC GLM. Values for percent body fat and percent body water were arcsine transformed. The same procedure was used to test differences in sex and season for total mass of food cache, total energy content of food cache, and number of days of energy stored in the food cache for houses from which food caches were recovered in the fall, winter, and spring. Tests for food cache variables were performed on ranked data. All multiple comparisons for seasons were made with unweighted means. Pearson's product moment correlation was used to test relationships among serum chemistry values, body composition values, and food cache variables.

RESULTS

Serum Chemistry

Plasma lipids showed seasonal fluctuations, but plasma proteins did not (Table 2). There were no significant effects of sex for any variable tested (Protein: F = 0.93, *d.f.* = 1, 27, P = 0.343; Triglycerides: $F = 0.001$, *d.f.* = 1, 27, $P = 0.974$; Cholesterol: $F = 0.01$, *d.f.* = 1, 27, $P = 0.931$), therefore sexes have been combined and are presented as seasonal means. There was not a significant difference in body mass between males and females ($F = 1.31$, *d.f.* = 1, 27, $P = 0.263$), however mean body mass of males was higher than that of females in every season except summer. Summer body mass was also significantly lower than spring body masses ($t = 3.06$, *d.f.* = 27, $P = 0.005$). Plasma protein concentrations were not different among seasons ($F = 1.33$, *d.f.* = 3, 27, $P = 0.285$). Triglyceride concentration did show a significant effect of season ($F = 5.89$, *d.f.* = 3, 27, $P = 0.003$). Triglycerides showed a sharp decrease from fall to winter ($t = 3.88$, *d.f.* = 27, $P = 0.001$), but were not significantly different from winter to spring ($t = 1.13$, *d.f.* = 27, $P = 0.267$) or from spring to summer ($t = 0.31$, *d.f.* = 27, $P = 0.758$). Lowest mean triglyceride concentrations occurred in the winter and highest in the fall. Serum cholesterol levels also showed a significant effect of season ($F = 41.38$, *d.f.* = 3, 27, $P < 0.0001$). Cholesterol declined significantly from fall to winter ($t = 4.84$, *d.f.* = 27, $P < 0.0001$) and from winter to spring ($t = 6.50$, *d.f.* = 27, $P < 0.0001$), but increased significantly from spring to summer ($t = 3.78$, *d.f.* = 27, $P = 0.001$). Body mass was not significantly correlated with triglycerides ($r = 0.11$, *d.f.* = 33, $P = 0.544$), cholesterol ($r = -0.24$, *d.f.* = 33, $P = 0.158$), or protein ($r = 0.07$, *d.f.* = 33, $P = 0.687$). Triglycerides and cholesterol were positively correlated ($r = 0.52$, *d.f.* = 33, $P = 0.001$).

Table 2. Seasonal changes in body mass and serum chemistry of eastern woodrats (*Neotoma floridana*). Values are presented as mean ± standard error.

Variable	Fall (*n* = 10)	Winter (*n* = 7)	Spring (*n* = 11)	Summer (*n* = 7)
Body mass (g)	250.0 ± 24.0	256.8 ± 14.8	280.4 ± 15.2	215.7 ± 15.4
Protein (g/dl)	6.74 ± 0.29	5.99 ± 0.22	6.34 ± 0.26	6.48 ± 0.19
Triglycerides (mg/dl)	88.75 ± 7.41	50.53 ± 5.23	57.81 ± 6.22	61.45 ± 2.63
Cholesterol (mg/dl)	257.6 ± 14.7	178.6 ± 12.2	71.5 ± 8.4	134.4 ± 12.2

Body Composition

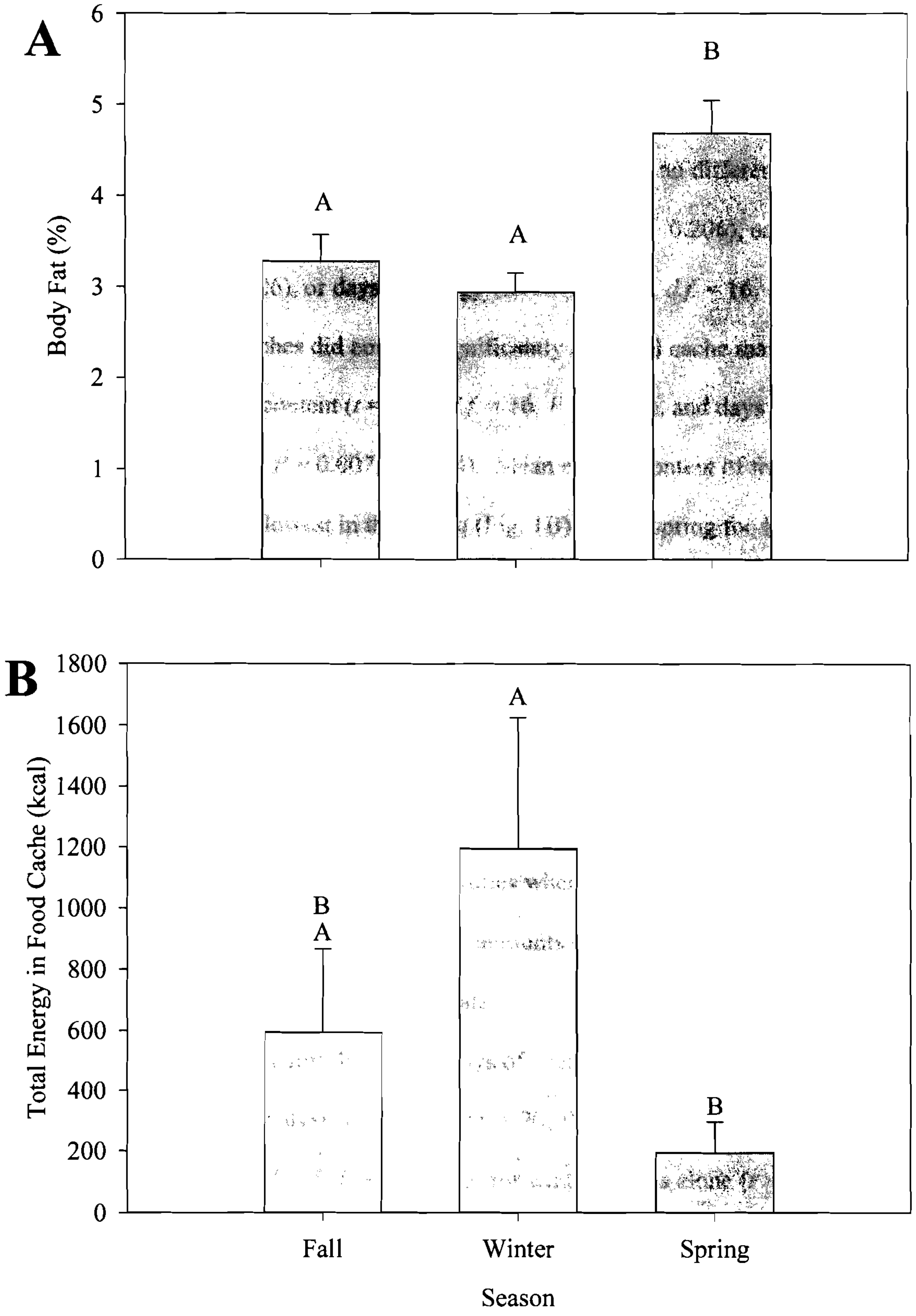
Eastern woodrats collected from the fall ($n = 10$), winter ($n = 7$), and spring ($n = 11$) were used in the body composition analysis. Body mass was not significantly different across these seasons ($F = 2.23$, *d.f.* = 2, 22, $P = 0.131$, Table 2). Lipid mass was significantly higher in males than females throughout the fall, winter, and spring ($F = 4.60$, *d.f.* = 1, 22, $P = 0.0433$, Table 3). Spring lipid mass was also higher than fall ($t = 4.08$, *d.f.* = 22, $P = 0.0005$) or winter lipid mass ($t = 4.00$, *d.f.* = 22, $P = 0.0006$). Lean dry mass was higher in males than females in each season as well ($F = 5.41$, *d.f.* = 1, 22, $P = 0.030$, Table 3). There was not a significant difference between seasons in lean dry mass ($F = 2.26$, *d.f.* = 2, 22, $P = 0.127$). Fat-free mass was higher in males than females in each season, but not significantly ($F = 4.11$, *d.f.* = 1, 22, $P = 0.055$). Fat-free mass also showed no difference between seasons ($F = 1.94$, *d.f.* = 2, 22, $P = 0.167$, Table 3). Percent body fat was significantly different between seasons ($F = 5.44$, *d.f.* = 2, 22, $P = 0.012$), but not sexes ($F = 0.02$, *d.f.* = 1, 22, $P = 0.895$). Percent body fat was not different in fall or winter ($t = 0.60$, *d.f.* = 22, $P = 0.557$), however it increased significantly in the spring ($t = 3.06$, *d.f.* = 22, $P = 0.006$, Fig. 1A). Percent body water also showed no difference between males and females ($F = 1.09$, *d.f.* = 1, 22, $P = 0.308$) and a significant difference for season ($F = 6.30$, *d.f.* = 2, 22, $P = 0.0009$). The only significant differences between seasons was that fall percent body water was higher than spring ($t = 3.55$, *d.f.* = 22, $P = 0.002$). Percent body water and percent body fat were negatively correlated ($r = -0.45$, *d.f.* = 26, $P = 0.017$). Lipid mass was positively correlated with body mass ($r = 0.56$, *d.f.* = 26, $P = 0.002$) and inversely correlated with serum cholesterol levels ($r = -0.61$, *d.f.* = 23, $P = 0.0006$).

Table 3. Seasonal values of lipid mass, lean dry mass, and fat-free mass for male and female eastern woodrats (*Neotoma floridana*). Values are mean ± standard error.

	Fall			Winter			Spring		
Variable	Male ($n = 6$)	Female ($n = 4$)	All ($n = 10$)	Male ($n = 4$)	Female ($n = 3$)	All ($n = 7$)	Male ($n = 2$)	Female ($n = 9$)	All ($n = 11$)
Lipid mass (g)	8.72	6.90	7.99	8.90	5.96	7.64	15.90	12.38	13.02
	± 1.69	± 1.55	± 1.03	± 1.02	± 0.78	± 0.86	± 0.80	± 1.17	± 1.05
Lean dry mass (g)	66.76	56.76	62.76	70.87	61.67	66.93	92.45	64.86	70.11
	± 9.14	± 10.95	± 6.82	± 5.92	± 4.03	± 3.97	± 13.76	± 3.01	± 5.01
Fat-free mass (g)*	254.45	223.36	242.01	263.58	229.82	249.12	330.76	253.29	266.31
	± 29.58	± 41.39	± 23.36	± 21.52	± 11.15	± 14.02	± 47.45	± 11.64	± 16.31

* Fat-free mass = body mass (including water) – lipid mass.

Figure 1. Seasonal change of energy stored as fat, represented as percent body fat (A), and energy stored as food, represented as total energy content of food caches in kcal (B), in eastern woodrats (*Neotoma floridana*) of east-central Kansas 1999-2000. Bars that share the same letter are not significantly different from each other at a significance level of $P < 0.05$.


A
Body Fat (%)
0
1
2
3
4
5
6
A
A
B
B
Total Energy in Food Cache (kcal)
0
200
400
600
800
1000
1200
1400
1600
1800
B
A
A
B
Fall
Winter
Spring
Season

Food Cache Analysis

Males and females did not differ in the mass of food cached ($F = 2.35$, *d.f.* = 1, 16, $P = 0.144$), energy content of the cache ($F = 2.80$, *d.f.* = 1, 16, $P = 0.114$), or the days of energy stored ($F = 2.82$, *d.f.* = 1, 16, $P = 0.206$). There was no difference between winter or fall caches in total cache mass ($t = 1.32$, *d.f.* = 16, $P = 0.206$), energy content ($t = 1.01$, *d.f.* = 16, $P = 0.326$), or days of energy stored ($t = 1.09$, *d.f.* = 16, $P = 0.293$). However, spring food caches did contain significantly less total cache mass ($t = 3.29$, *d.f.* = 16, $P = 0.005$), energy content ($t = 3.17$, *d.f.* = 16, $P = 0.006$), and days of energy stored ($t = 3.08$, *d.f.* = 16, $P = 0.007$, Table 4). Mean energy content of food caches was highest in the winter and lowest in the spring (Fig. 1B). Most spring food caches were observed to be almost entirely consumed on retrieval.

Dicot leaf material was the only food type that was found in all caches in the fall, winter, and spring (Table 5). Foliage of red cedar was a larger component of food caches in the winter and spring than in the fall. High energy food types, such as black walnut and rough-leaved dogwood (*Cornus drummondii*), were not found frequently in fall and winter caches; they were found in large quantities when present in a cache (Table 5). There was a high amount of variability in the amounts of food stored and the types of food stored among individual eastern woodrats.

Body mass was not correlated with days of energy stored when all seasons' caches were included in analysis ($r = -0.33$, *d.f.* = 20, $P = 0.132$), for analysis of fall caches alone ($r = -0.69$, *d.f.* = 5, $P = 0.089$), or for winter caches alone ($r = 0.28$, *d.f.* = 4, $P = 0.595$). Also, body mass was not correlated with total energy content of the cache ($r = -0.25$, *d.f.* = 20, $P = 0.266$) or with total cache mass ($r = -0.23$, *d.f.* = 20, $P = 0.295$) for

Table 4. Seasonal characteristics of eastern woodrat (*Neotoma floridana*) food caches in east-central Kansas. Values are presented as mean ± standard error.

Characteristic	Fall (*n* = 7)	Winter (*n* = 6)	Spring (*n* = 9)
Total mass of cache (g)	121.96 ± 49.62	261.68 ± 88.44	41.89 ± 21.51
Energy content of cache (kcal)	593.2 ± 273.3	1194.9 ± 428.9	194.0 ± 102.0
Days of energy stored	18.6 ± 8.8	32.0 ± 11.4	5.3 ± 2.8

Table 5. Seasonal composition of eastern woodrat (*Neotoma floridana*) food caches in east-central Kansas, 1999-2000.

Plant Type	Percent of total cache (g dry mass)	Range (g dry mass)	Frequency of occurrence in caches
Fall (n = 7)			
Dicot leaf material	57.98	9.5 – 240.7	7
Rough-leafed dogwood fruit	35.68	0.0 – 304.6	1
Red cedar foliage	2.85	0.0 – 18.6	3
Chewed sticks	2.4	0.0 – 15.5	3
Grass	1.04	0.0 – 4.6	4
Kentucky coffee-tree (seeds)	0.05	0.0 – 0.4	1
Coralberry	0.05	0.0 – 0.4	1
Winter (n = 6)			
Dicot leaf material	62.25	4.7 – 360.6	6
Black Walnut (nut)	11.81	0.0 – 182.8	2
Red cedar foliage	10.96	0.0 – 93.1	4
Rough-leafed dogwood fruit	4.31	0.0 – 67.7	1
Fungi	4.17	0.0 – 65.4	1
Prickly pear cactus	3.87	0.0 – 54.7	2
Honey locust (seeds)	1.85	0.0 – 29.1	1
Grass	0.78	0.0 – 3.3	5
Spring (n = 9)			
Red cedar foliage	41.88	0.0 – 84.6	5
Dicot leaf material	22.07	0.6 – 45.3	9
Honey locust (seeds)	18.33	0.0 – 69.1	1
Osage orange (seeds)	14.30	0.0 – 53.9	1
Black Walnut (nut)	1.70	0.0 – 5.4	2
Chewed sticks	1.67	0.0 – 5.3	2
Grass	0.05	0.0 – 0.2	1

all individuals or within the fall and winter seasons. Days of energy stored in fall caches ranged from 1.6 for a 349 g eastern woodrat to 63.4 days for a 192 g eastern woodrat. Days of energy stored in winter caches ranged from 2.7 days for a 257 g eastern woodrat to 69.4 days for a 270 g eastern woodrat. Only three of nine spring food caches had more than two days of energy remaining in the cache and only one had more than 11 days of energy remaining.

House volumes did not differ between males and females ($F = 0.85$, *d.f.* = 1, 22, $P = 0.366$) and body mass was not correlated with house volume ($r = -0.38$, *d.f.* = 24. $P = 0.052$). In addition, house volume was not correlated with the total mass of the food cache at any study site ($r = -0.21$, *d.f.* = 20, $P = 0.480$). Mean house volume was 0.62 ± 0.49 m^3. No relationships were found between food cache variables (total cache mass, energy content of cache, days of energy stored, or ADER) and serum chemistry values (triglycerides, cholesterol, or protein concentrations) within seasons or with all individuals included in analysis.

Fat Stores vs. Food Stores

Energy stored as fat was lowest in the winter when energy stored as cached food was highest and energy stored as fat was highest in the spring when energy stored as cached food was lowest (Fig. 1). Intermediate levels of fat stores and food stores occurred in the fall. In the fall, percent body fat was positively correlated with total cache mass ($r = 0.83$, *d.f.* = 5, $P = 0.021$), energy content of the cache ($r = 0.80$, *d.f.* = 5, $P = 0.032$), and days of energy stored ($r = 0.81$, *d.f.* = 5, $P = 0.028$). However, percent body fat was negatively correlated with ADER ($r = -0.91$, *d.f.* = 5, $P = 0.005$) and body mass ($r = -0.90$, *d.f.* = 5, $P = 0.006$) in the fall. Percent body fat was not correlated with any of these variables in the winter or in the spring ($P > 0.05$). When fall and winter cached food data were combined, percent body fat was positively correlated with total cache mass ($r = 0.58$, $d.f. = 11$, $P = 0.037$), energy content of the cache ($r = 0.61$, $d.f. = 11$, $P = 0.026$), and days of energy stored ($r = 0.64$, $d.f. = 11$, $P = 0.018$), however the negative correlations with ADER ($r = -0.48$, $d.f. = 11$, $P = 0.097$) and body mass ($r = -0.47$, $d.f. = 11$, $P = 0.108$) were no longer significant. In the spring, percent body fat was not significantly correlated with any food cache variable (total cache mass: $r = 0.048$, $d.f. = 7$, $P = 0.903$; energy content of cache: $r = 0.049$, $d.f. = 7$, $P = 0.900$; days of energy stored: $r = 0.047$, $d.f. = 7$, $P = 0.911$; ADER: $r = -0.076$, $d.f. = 7$, $P = 0.846$).

DISCUSSION

Plasma lipids (triglycerides and cholesterol) are commonly used measures of fat metabolism (Seal et al. 1972). Large quantities of triglycerides appear during conditions when fat is being used for energy (Guyton 1976). In my study, eastern woodrats showed high levels of plasma lipids in the fall, which corresponds to the time when caching activity is most pronounced. Such high plasma lipids likely reflect the mobilization of fat reserves to account for the energetic cost of caching. However, high lipid levels may also potentially reflect the consumption of high lipid foods. Plasma lipids were at their lowest levels in the winter, which indicated a highly reduced amount of fat metabolism occurring at that time relative to the fall. Spring triglyceride concentrations increased from the winter, but the increase was not drastic. However, cholesterol continued to significantly decrease in the spring. Cholesterol levels decrease with increased production of estrogens and androgens in humans (Guyton 1976). The breeding season of eastern woodrats in Kansas begins in February (Rainey 1956) and spring individuals in my study were collected in March. The spring sample included a disproportionate number of females ($n = 9$) to males ($n = 2$), which suggested that the effects of estrogens may account for the continued decline of cholesterol in the spring.

Similar patterns of serum cholesterol were observed for white-tailed deer (*Odocoileus virginianus*, Coblentz 1975, Warren et al. 1981). Coblentz (1975) suggested that the observed decrease in winter cholesterol levels was due to a reduction in dietary quality. Warren et al. (1981) were not able to determine if reductions in winter cholesterol in their study was due to nutritional effects or reproductive effects, but did suggest that Coblentz's (1975) pattern was likely due to reproductive effects and not a nutritional effect. Hellgren et al. (1993) found that triglyceride concentrations of black bears were highest in the autumn and during hibernation, corresponding to times when mobilization of fat reserves would be expected to be highest. In addition, Harlow and Seal (1981) found a 34% increase in triglycerides at day 20 of fasting in badgers.

Plasma protein concentrations in eastern woodrats remained constant across study seasons. A constant equilibrium exists between plasma proteins, amino acids in the blood, and tissue proteins (Guyton 1976). Proteins of tissues are not degraded for use as energy until the majority of carbohydrates and fat stores in the body have been used, as would occur during starvation (Guyton 1976), which is important to animals that must survive prolonged periods of time during food scarcity or with no food at all. The badger minimizes protein catabolism during fasting by mobilizing fat stores throughout the period of starvation (Harlow and Seal 1981). The eastern woodrats in my study were not experiencing any extreme nutritional stress (i.e. starvation), as indicated by the constant plasma protein concentrations.

Energy stored as food in the cache was at its highest in the winter (Fig. 1B), however mean seasonal energy contents were much lower than those reported elsewhere. Post et al. (1993) reported a mean energy content of fall caches of 3,682 ± 53 kcal while winter caches contained 2,369 ± 22 kcal. Horne et al. (1998) reported mean energy contents of caches taken from artificial houses of 6,808.6 ± 637.1 kcal in the winter and 2,448.3 ± 660.6 kcal in the spring. Both of these studies were conducted in central Kansas. The highest mean energy content of caches in my study was in the winter and was half those reported by Post et al. (1993) and Horne et al. (1998). The dominant high-energy food in previous studies was rough-leaved dogwood fruit (Post et al. 1993) and Osage orange (Horne et al. 1998). These food types did occur in caches that I collected, but not frequently. The only common food type that occurred in caches of my study was dicot leaf material. In addition, red cedar foliage was cached in small amounts in the studies by Post et al. (1993) and Horne et al. (1998). Red cedar makes up a large portion of the winter diet of eastern woodrats in Kansas, however it is not typically cached in large amounts because it is readily available throughout the winter (Post 1991). Eastern woodrats in my study cached red cedar frequently and in relatively large quantities (Table 5), which suggested that the availability of food might have been low and thus resulted in the caching of a food type that ordinarily would not have been cached. The year of my study was a drought year and that may account for the seemingly lower availability of food in the winter of 1999-2000. Fitch and Rainey (1956) stated that Osage orange is the single most important plant species to eastern woodrats for its food provided by leaves and seeds. Over 75% of the dicot leaves collected from eastern woodrat caches were Osage orange. Eastern woodrats commonly build their houses at the base of Osage orange trees and such was the case for the eastern woodrats trapped in my study. The estimates of energy content of the caches presented here are likely low compared to the energy actually available. Due to the large size of Osage orange fruits, they are not commonly stored in the caches of eastern woodrats, however they were abundant around houses. The high-calorie seeds of Osage orange fruits in the vicinity of eastern woodrat houses at my study sites were most certainly being consumed throughout the winter and are an important resource that was not included in food cache energy values. Another assumption that affects energy estimations of caches is that all of the edible portion of the cache is digestible (Post et al. 1993).

I did not find a relationship between body mass of eastern woodrats and days of energy stored in the cache, which agreed with the findings of Horne et al. (1998), but not with Post et al. (1993), who found that larger eastern woodrats had disproportionately more days of energy stored in their caches than did smaller eastern woodrats. My study agreed with both of the two previous studies in finding no differences between males and females in house volume, cache mass, energy content of the cache, or number of days of energy stored. House volumes were not related to body mass in eastern woodrats studied here, which was consistent with the findings of Post et al. (1993). However, Horne et al. (1998) found a positive correlation between body mass and house volume in eastern woodrats. I found that house volume was not correlated with total cache mass, as did Post et al. (1993), which may be explained by the smaller cache sizes in my study.

Energy stored as fat (% body fat) and energy stored as food (energy content of the cache) were at intermediate levels in the fall. In the winter, eastern woodrats maximized their external energy stores while internal energy stores were at a minimum (Fig. 1). Consequently, internal energy stores were maximized in the spring and external energy stores were at their lowest level. Such a pattern suggested a strategy of eastern woodrats to obtain most of their needs in the winter from the food cache and to conserve fat stores during that time in order to survive the winter and possibly to maximize internal energy stores in the spring. Support for this was also seen from the lower plasma lipid levels in the winter, which showed a low level of fat mobilization. The potential benefit of such a strategy is most likely related to spring breeding. By maximizing internal energy stores in the spring, an eastern woodrat would maximize the amount of energy available for reproduction in the spring. Fat stores would be more beneficial than food stores at the beginning of the breeding season for several reasons. First, nutrient concentration of cached foods of eastern woodrats can decline over time (Post 1992). Food stores that remain in the spring may no longer be as much of a benefit to the cache owner as they would be in the winter if lipid content of the food had declined. Post (1992) found that the largest decrease in nutritional content was in the lipid content of rough-leaved dogwood fruits. Second, having most of the spring energy stores as body fat minimizes the amount of time spent handling and consuming cached items or time spent foraging for additional food, which would allow more time to be spent searching for mates and breeding. Although eastern woodrats obtained most of their winter energy supply from the food cache, they did obtain parts of their winter diet from outside of the cache (Post 1991). Finally, maximizing internal energy stores at the onset of the breeding season may benefit early breeders. Breeding early would allow the possibility of breeding more often and females could have more litters during the breeding season, which would increase fitness. Eastern woodrats have two to three litters annually, most in the spring, and litters may follow in succession because females mate soon after giving birth (Wiley 1980). Therefore, having a large internal energy store available in the spring would increase a females chances of having multiple litters during the breeding season.

Little work has been done on the relationship between food stores and fat stores in hoarding animals (Vander Wall 1990). An exception was the study of Wrazen and Wrazen (1982) with eastern chipmunks, which provided some comparison for the results found here. Eastern chipmunks are larder hoarders and stored food is essential for winter survival (Brenner and Lyle 1975). Eastern chipmunks also showed a wide variation in winter hibernation: some captives were torpid, some were semi-torpid, some were occasionally semi-torpid, and some remained active (Panuska 1959). Wrazen and Wrazen (1982) studied the relationship between hoarding, body mass, and torpor in eastern chipmunks and found three potential survival strategies, including: 1) individuals that maintained smaller caches, gained mass before winter, and became semi-torpid, 2) individuals that maintained large caches, did not gain mass, and became torpid, and 3) individuals that maintained large caches, did not gain body mass, and remained active. They suggested that there was a balance between internal and external storage of energy and that the first strategy would result in a spring surplus of internal storage, the second a surplus of external storage, and the third in the depletion of all energy stores. Wrazen and Wrazen (1982) related their findings to spring breeding as well, indicating that individuals who remained active would increase their probability of breeding early. Eastern woodrats remain active throughout the year and are nocturnal, captives spend most of the day sleeping (Wiley 1980). Torpor has not been demonstrated in eastern woodrats. The strategy of eastern woodrats for winter survival found in my study most resembled the third strategy found by Wrazen and Wrazen (1982) for eastern chipmunks. However, Wrazen and Wrazen (1982) predicted that the third strategy would lead to depletion of all energy stores, which was not the case in eastern woodrats. Probably periodic arousal from torpor depleted more internal energy stores than remaining active and using a large cache for energy during the winter. The benefit of a large cache and minimizing activity seemed to result in a conservation of fat stores during the winter in eastern woodrats, which resulted in internal energy benefits in the spring. The spring condition of eastern woodrats (high internal energy stores) would have been predicted by the first strategy of Wrazen and Wrazen (1982, small cache, gain mass in winter, and become semi-torpid). This was not supported by my findings, which showed that eastern woodrats store large caches, gain mass in the spring, and remain active. The fact that eastern chipmunks go into torpor and eastern woodrats do not likely has a large effect on the strategies and outcomes of these strategies in the two species.

When relationships were tested within the fall, percent body fat was negatively correlated with body mass, which suggested that smaller eastern woodrats had a higher proportion of body fat. Although body mass was not correlated with cache mass in the fall, the relationship approached significance ($r = -0.69$, $P = 0.089$). Percent body fat was positively correlated with cache mass in the fall, which indicated that smaller eastern woodrats with high percent body fat also had more food stored. These relationships may provide evidence that larger eastern woodrats can wait until later in the fall to begin caching large quantities of food, thereby delaying the cost of defending the food cache. If one assumes that larger eastern woodrats are older and more experienced cachers, the relationship is a possibility.

Eastern woodrats demonstrated a definite pattern in their use of energy stored as fat and food. Winter was characterized by high food stores relative to fat stores, apparent conservation of fat stores in the winter, and a surplus of fat stores in the spring. Serum chemistry analysis agreed with the pattern, showing lowest levels of lipid mobilization in the winter. Such a strategy would have apparent benefits in available energy for reproduction in the spring. In such a case, the larder hoarder benefits from the cost of obtaining and defending its larder by a surplus of internal energy for reproduction in the spring, thereby maximizing the efficiency of converting resources into offspring. Further research into the reproductive benefit of high spring internal energy stores is necessary, as well as the effect of food availability on the relationship between internal and external energy stores in caching species.

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CHAPTER FOUR

THE USE OF TOTAL BODY ELECTRICAL CONDUCTIVITY (TOBEC) TO PREDICT LEAN AND LIPID MASS IN EASTERN WOODRATS (NEOTOMA FLORIDANA)

ABSTRACT: Total body electrical conductivity (TOBEC) has become an increasingly used method of estimating lean and lipid mass in small birds and mammals. The TOBEC value of an animal is highly correlated with lean mass. Prediction of lean mass with a linear regression equation allows for estimation of lipid mass by subtracting lean mass from total body mass (two-stage model). The accuracy of two-stage models for predicting lipid mass has been questioned by previous investigators, who suggested that lipid mass was more accurately predicted directly from body mass and TOBEC (direct model). I examined the accuracy of TOBEC for predicting lipid mass in eastern woodrats (Neotoma floridana). Four two-stage models were tested. The models varied in the use of TOBEC as the independent or dependent variable and in the use of a transformed TOBEC value. Three direct models were tested in which lipid mass was predicted from TOBEC value or transformed TOBEC value and body mass. The direct models performed much better than two-stage models in predicting lipid mass, with a relative error in predicted lipid mass of 80% lower than the best two-stage model. There was little difference in predictive error between two-stage models that used TOBEC as the independent or dependent variable. The TOBEC value transformed for body geometry did not greatly improve either the two-stage or direct models. TOBEC showed potential in the prediction of lipid mass in eastern woodrats and was the most accurate non-invasive method of determining lipid mass available.

Key words: eastern woodrat, lipid estimation, lipids, Neotoma floridana, TOBEC, total body electrical conductivity.

INTRODUCTION

Total body electrical conductivity (TOBEC) has received much attention from biologists as a method for predicting lean and lipid mass of small birds and mammals. TOBEC is superior to traditional methods of determining lipid mass because it is non-invasive, meaning it does not require killing the subject animal. In addition to being more humane, TOBEC would allow for body composition studies of individuals over time, which is not possible with lipid determination by chemical extraction. The potential application of TOBEC to ecological studies was first introduced by Walsberg (1988). TOBEC works on the principle that the placement of an animal within a coil producing a stable electromagnetic field causes a change in the impedance of the surrounding coil, related to the electrical conductivity of the sample. Because the electrical conductivity of lipid mass is less than 5% of lean mass, the conductive index obtained by TOBEC is highly correlated with lean mass (Pethig 1979). Subtraction of TOBEC-predicted lean mass (also termed fat-free mass when lipid determination is by chemical extraction) from body mass provides an estimate of lipid mass. Initial interspecific studies with TOBEC reported high coefficients of determination, R^2, between lean mass and TOBEC values (Castro et al. 1990; Walsberg 1988).

Morton et al. (1991) stated that in TOBEC applications, a high R^2 value for lean mass regressions misrepresented the precision with which lipid mass was estimated. Although the absolute error of predicting lean and lipid mass was identical, the relative error of predicted lipid mass was much greater because lipid mass is typically a much smaller proportion of body mass than that of lean mass. Morton et al. (1991) suggested that directly predicting lipid mass from TOBEC values and body mass (a direct model) worked better than predicting lean mass and determining lipid mass by subtraction from total body mass (a two-stage model). In addition, Scott et al. (1991) suggested that intraspecific predictive equations were more appropriate for use with TOBEC and Roby (1991) suggested that a linear regression model was only appropriate over a narrow range of lean body mass and that prediction equations need to be determined for each species of interest. Subsequent TOBEC studies have generated species-specific predictive equations and have compared the performance of the direct and two-stage models (Asch and Roby 1995; Burger 1997; Conway et al. 1994; Lyons and Haig 1995; Skagen et al. 1993; Spengler et al. 1995; Voltura and Wunder 1998). Where applicable, direct models have typically included morphological measures as independent variables, along with TOBEC values and body mass.

Predictive models of lean mass have also varied in using the TOBEC value as the dependent variable (Asch and Roby 1995; Castro et al. 1990; Morton et al. 1991; Roby 1991; Skagen et al. 1993; Voltura and Wunder 1998; Walsberg 1988) or as the independent variable (Bachman 1994; Burger 1997; Conway et al. 1994; Lyons and Haig 1995; Scott et al. 1991; Spengler et al. 1993). Castro et al. (1990) suggested that using TOBEC as the independent variable is inappropriate because it assumes that TOBEC is measured without error, however there is likely less error associated with the chemical determination of lean mass than there is in the measurement of TOBEC. Scott et al. (1991) disagreed with the statistical approach of Castro et al. (1990) and suggested that TOBEC was measured with high reproducibility if proper attention was paid to the positioning of the animal in the measurement chamber.

TOBEC can be measured with a variety of models of the Small Animal Body Composition Analyzer manufactured by EM-SCAN®, Inc. (Springfield, Illinois). The TOBEC value (also referred to as EM-SCAN number) can be transformed for total body length of the animal because of the effect of body geometry, as recommended by EM-SCAN®, Inc. (EM-SCAN, Inc. 1993) and first described by Fiorotto et al. (1987). Such a transformation was used by Scott et al. (1991) and Lyons and Haig (1995) with species of shorebirds and Voltura and Wunder (1998) with prairie voles (Microtus ochrogaster).

Although TOBEC has been used extensively in studies with birds, it has received limited use in studies with mammals. Walsberg (1988) used a number of species of small mammals to determine an interspecific equation for mammals. However, only Bachman (1994), with Belding's ground squirrel (Spermophilus beldingi), and Voltura and Wunder (1998), with the prairie vole, have used TOBEC in a species-specific study of a wild mammal species. Voltura and Wunder (1998) suggested that TOBEC can be useful for predicting lean mass, but it was not accurate in predicting lipid mass of mammals that remain relatively lean throughout the year, such as prairie voles. However, eastern woodrats are larger then prairie voles and therefore should carry a greater lipid mass. As part of a body composition study of eastern woodrats (Neotoma floridana) in east-central Kansas, TOBEC predictive equations of lean and lipid mass were determined with the EM-SCAN SA-3000 Small Animal Body Composition Analyzer. The eastern woodrat is a medium-sized rodent that typically ranges in body mass from 150 g to 350 g (Wiley 1980). In order to evaluate the performance of the predictive equations for future use, a variety of direct and two-stage models were tested. The objectives of my analysis were:

1) to determine if a direct model or a two-stage model performed better at predicting lipid mass in eastern woodrats, 2) to determine the effect of using TOBEC value as the independent or dependent variable on two-stage models, and 3) to determine if the use of a TOBEC value transformed for body geometry improved the performance of the model in predicting lipid mass.

MATERIALS AND METHODS

Twenty-eight eastern woodrats were collected from Chase, Coffey, and Lyon counties, Kansas, between 20 October 1999 and 14 March 2000. Eastern woodrats were anesthetized with 0.22 to 0.26 mg/ml of ketamine (Fort Dodge Laboratories, Fort Dodge, Iowa), depending on body size, to immobilize the animal while in the measurement chamber. Once immobilized, body mass and sex were determined and the average of five readings taken in the EM-SCAN SA-3000 was used as the TOBEC value for each eastern woodrat. Body geometry influences TOBEC, therefore each eastern woodrat was consistently placed in the chamber in the same position with the tail tucked under the body. After EM-SCAN readings were obtained, approximately 3 ml of blood were obtained by heart puncture, for use in a separate study, and the eastern woodrat was killed by cervical dislocation following the guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998), placed in a plastic bag, and frozen until later analysis.

Before body composition determinations were made, carcasses were thawed, weighed, and total body length, length of the right hind foot, and zygomatic breadth were recorded. Carcasses were dried at 80°C in a drying oven (Kerr et al. 1982) until the carcass reached constant mass. Carcasses were ground in a coffee grinder and the entire homogenized sample was sent to the Department of Zoology, University of Western Ontario, London, Ontario, Canada for chemical extraction. Cellulose thimbles were dried to constant mass (thimble) and depending on thimble size, were then filled with approximately 10 or 40 g of the dry, ground carcass. Thimbles and samples were dried to constant mass (thimble + sample dry mass) and then extracted with petroleum ether

(Dobush et al. 1985) in a modified Soxhlet apparatus. Following extraction of lipids, thimbles and lean samples were returned to the ovens and dried to constant mass (thimble + lean sample dry mass). Fat content was calculate as:

$$\text{Total carcass fat} = \frac{\text{sample fat}}{\text{sample dry mass}} \times \text{total carcass dry mass}$$

$$\text{or} \quad \frac{(\text{thimble} + \text{sample mass}) - (\text{thimble} + \text{lean sample dry mass})}{(\text{thimble} + \text{sample dry mass}) - (\text{thimble})} \times \text{total carcass dry mass.}$$

Chemical extractions were performed on the entire dry sample of the carcass. Petroleum ether is preferred for studies of body composition because it extracts lipids completely but does not extract nonlipid material (Dobush et al. 1985). Fat-free mass was calculated as the total body mass of the individual at the time of capture minus the lipid mass determined by chemical extraction. Lipid was also expressed as a percentage of total body mass.

One individual was excluded from further analysis because it lacked a tail, therefore its body geometry was altered compared to the other eastern woodrats. The average TOBEC value for each individual was transformed for individual body length with the equation:

$$\text{transformed TOBEC value} = (\text{TOBEC value} \times \text{Total body length})^{-0.5}.$$

All regression models were performed with least squares regression. Seven linear regression models were used to predict lipid mass. The first four models were two-stage models, that is fat-free mass was predicted and subtracted from body mass to obtain lipid mass. In models 1 and 2, fat-free mass was the independent variable and TOBEC value or transformed TOBEC value was the dependent variable. In these models, the regression equation was algebraically rearranged to solve for fat-free mass. In models 3 and 4, fat-free mass was used as the dependent variable with the TOBEC value or the transformed TOBEC value used as the independent variable. These models were used to compare the performance of TOBEC value as the dependent and independent variable.

The remaining models were direct models with lipid mass as the dependent variable. Stepwise linear regression was used to determine what combination of body mass, TOBEC value or transformed TOBEC value, total body length, right hind foot length, and zygomatic breadth best predicted lipid mass. A significance level of $P = 0.05$ was set for a variable to be included into the model and to remain in the model. The results of the stepwise regression showed that no variable other than body mass and TOBEC value or transformed TOBEC value improved the prediction of lipid mass, therefore the morphological measure, with the exception of body mass, were not included in any further models. Models 5 and 6 were multiple regression models that used lipid mass as the dependent variable and TOBEC value (model 5) or transformed TOBEC value (model 6) and body mass as independent variables. In order to determine how well lipid mass could be determined without TOBEC, model 7 predicted lipid mass directly from body mass. Following Lyons and Haig (1995) and Voltura and Wunder (1998), R^2, mean square error (MSE), width of the 95% confidence interval, and absolute and relative error were used as criteria to determine which model best predicted lipid mass. Absolute error was calculated as the absolute value of the predicted value minus observed value. Relative error was calculated as the absolute error divided by the observed value and was multiplied by 100 to be expressed as a percentage.

Based on the above criteria, the best two-stage and direct models were chosen. The "best" model was considered to be the model that had the highest R^2, lowest MSE, smallest 95% confidence interval, and the lowest absolute and relative error in predicted lean and lipid mass. Ten of the 27 eastern woodrats were randomly chosen to be part of a validation set. These woodrats were excluded from the data set and predictive equations of the two best models were determined with the remaining 17 individuals. Lipid mass was then predicted by using the best two-stage and direct models for the ten randomly chosen eastern woodrats. Predicted lipid mass from each model was then regressed on actual lipid mass with least squares regression. All statistical analyses were performed with the Statistical Analysis System (SAS Institute, Inc. 1999).

RESULTS

All body composition and morphological values are presented as mean ± standard deviation with range in parentheses. Mean body mass of the 27 eastern woodrats included in the study was 262.49 ± 59.01 g (139.00 – 378.20). Mean fat-free mass was 252.85 ± 56.98 g (133.34 – 378.20) and mean lipid mass was 9.64 ± 3.83 g (4.37 – 17.79). Percent body fat averaged 3.74 ± 1.21 % and mean body water was 70.76 ± 1.38 %. Mean morphological measurements were 332.2 ± 23.0 mm (320.0 – 382.0) for total body length, 37.7 ± 1.36 mm (35.0 – 41.0) for right hind foot length, and 25.7 ± 1.55 mm (22.6 – 29.5) for zygomatic breadth.

Values for R^2, MSE, width of the 95% confidence interval, absolute error and relative error of predicting fat-free and lipid mass for each model are presented in Table 1. All four two-stage models predicted fat-free mass well, with R^2 values greater than 0.96. When fat-free mass was the independent variable (models 1 and 2), the transformed TOBEC value did not greatly improve any of the criteria with the exception of MSE, which was reduced from 457.91 to 140.27. In models 4 and 5, where fat-free mass was the dependent variable, the transformed TOBEC value did not greatly improve any of the model criteria. There was also not a large difference in any of the two-stage model criteria whether fat-free mass was used as the independent or dependent variable (Table 1). All four two-stage models poorly predicted lipid mass with relative errors of greater than 100%. Model 4 was chosen as the best two-stage model based on a high R^2 and the lowest error values of all the indirect models. Model 4 predicted fat-free mass with the following equation:

Table 1. Comparison of model criteria for two-stage and direct regression models estimating lean and lipid mass in eastern woodrats (Neotoma floridana) with total body electrical conductivity (TOBEC). Values are presented as mean ± standard error.

Model[a,b]	R^2	MSE	Width of 95% confidence interval	Absolute error of lean mass (g)	Relative error of lean mass (%)	Absolute error of lipid mass (g)	Relative error of lipid mass (%)
Model 1: T (dep) vs. FFM (ind)	0.962	457.91	12.39 ± 3.79	9.11 ± 6.47	3.77 ± 2.63	9.11 ± 6.47	109.40 ± 88.75
Model 2: TT (dep) vs. FFM (ind)	0.965	140.27	11.87 ± 3.63	8.30 ± 6.77	3.38 ± 2.72	8.30 ± 6.77	105.37 ± 106.04
Model 3: FFM (dep) vs. T (ind)	0.962	128.16	12.18 ± 3.65	8.93 ± 6.35	3.77 ± 2.93	8.93 ± 6.35	108.20 ± 91.28
Model 4: FFM (dep) vs. TT (ind)	0.965	117.87	11.63 ± 3.65	8.33 ± 6.43	3.32 ± 2.46	8.33 ± 6.43	104.39 ± 103.22
Model 5: LM (dep) vs. BM and T (ind)	0.425	9.14	3.96 ± 1.30	NA	NA	2.14 ± 1.92	24.84 ± 22.02
Model 6: LM (dep) vs. BM and TT (ind)	0.407	9.41	4.05 ± 1.21	NA	NA	2.15 ± 1.97	24.16 ± 24.13
Model 7: LM (dep) vs. BM (ind)	0.311	10.51	3.48 ± 1.06	NA	NA	2.34 ± 2.10	26.86 ± 26.30

[a] dep = dependent variable, ind = independent variable.

[b] T = TOBEC value, TT = transformed TOBEC value, FFM = fat-free mass, LM = lipid mass, BM = body mass.

Fat-free mass = 0.90 (transformed TOBEC value) – 52.10.

The direct multiple regression models (models 5 and 6) significantly predicted lipid mass (model 5: $\underline{R}^2 = 0.425$, d.f. = 25, $\underline{P} = 0.001$; model 6: $\underline{R}^2 = 0.407$, d.f. = 25, $\underline{P} = 0.002$). transformed TOBEC value did not improve any of the model criteria for the direct model. Model 5 was the best direct model and it predicted lipid mass with the following equation:

Lipid mass = 0.14 (body mass) – 0.06 (TOBEC value) – 6.55.

Absolute error in predicted lipid mass was 8.33 ± 6.43 g for model 4 (the two-stage model) and 2.14 ± 1.92 g for model 5 (the direct model), a reduction of 74% in absolute error with the direct model. Furthermore, relative error in predicting lipid mass was 80% lower with the direct model compared to the two-stage model.

Body mass alone significantly predicted lipid mass ($\underline{R}^2 = 0.311$, d.f. = 25, $\underline{P} = 0.003$). Inclusion of TOBEC value into the direct model explained 11.3% more of the variation in lipid mass, decreased MSE from 10.50 to 9.14, and decreased the relative error in predicting lipid mass by approximately 2%.

Actual lipid mass did not significantly estimate lipid mass predicted by model 4 for the 10 eastern woodrats included in the validation set ($\underline{R}^2 = 0.115$, d.f. = 8, $\underline{P} = 0.338$, Fig. 1). However, lipid mass predicted by the direct model (model 5) was significantly related to actual lipid mass ($\underline{R}^2 = 0.736$, d.f. = 8, $\underline{P} = 0.002$, Fig. 2).

Fig. 1. Relationship between actual lipid mass and the lipid mass predicted by the two-stage model (model 4) for the 10 eastern woodrats (Neotoma floridana) included in the validation set.

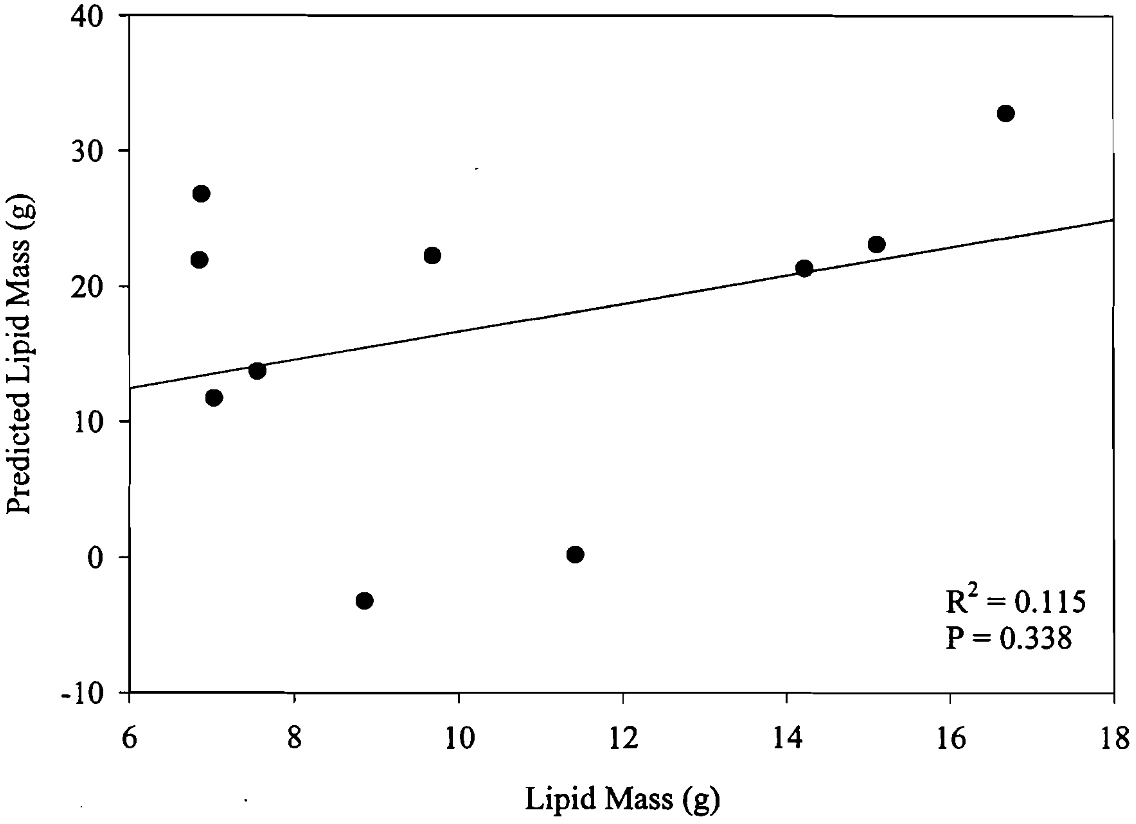
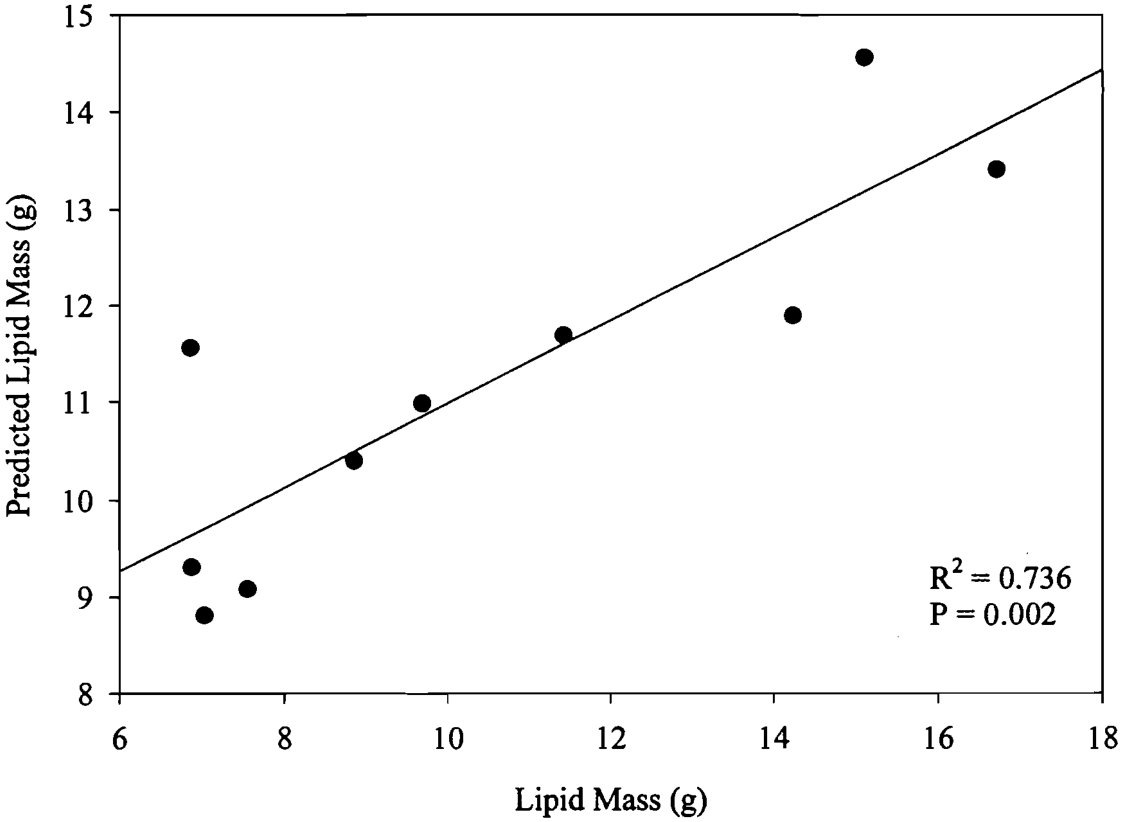

40
30
20
10
0
-10
Predicted Lipid Mass (g)
6
8
10
12
14
16
18
Lipid Mass (g)
$R^2 = 0.115$
P = 0.338

Fig. 2. Relationship between actual lipid mass and the lipid mass predicted by the direct model (model 5) for 10 eastern woodrats (Neotoma floridana) included in the validation set.


Predicted Lipid Mass (g)
15
14
13
12
11
10
9
8
6
8
10
12
14
16
18
Lipid Mass (g)
R² = 0.736
P = 0.002

DISCUSSION

As with most studies of body composition, my variable of primary interest was lipid mass. Therefore, predictive models including TOBEC needed to be evaluated based on how well they estimated lipid mass. In my study, two-stage models predicted fat-free mass of eastern woodrats with a high level of accuracy comparable to previous studies with birds and mammals (Bachman 1994; Castro et al. 1990; Roby 1993; Skagen et al. 1993; Voltura and Wunder 1998; Walsberg 1988). However, two-stage models poorly predicted lipid mass in eastern woodrats. Average relative error in predicted lipid mass of the four two-stage models tested was 107%. In addition, actual lipid mass of the validation set only explained 11.5% of the variation in the lipid mass predicted for these eastern woodrats by the best two-stage model. These results were in agreement with those of Morton et al. (1990), who first discussed the potential problems in predicting lipid mass with two-stage models. In my study, direct models in which lipid mass was predicted directly from body mass and TOBEC value estimated lipid mass of eastern woodrats with a much higher level of accuracy than did two-stage models. The best direct model predicted lipid mass with a relative error of 24.84%, which was 79.55% better than the best two-stage model. In addition, actual lipid mass of eastern woodrats included in the validation set explained 73.6% of the variation in lipid mass predicted by the direct model for these individuals. The inclusion of TOBEC in direct models improved the accuracy with which lipid mass was predicted in eastern woodrats by 4% and explained 11% more of the variance in lipid mass (Table 1).

There has been considerable debate about the use of TOBEC as the independent or dependent variable in two-stage models (see Burger 1997; Castro et al. 1990; Scott et al. 1991). In my study, there was little difference between two-stage models that used TOBEC values as the independent or dependent variable. Models 3 and 4, which used TOBEC as the independent variable had the lowest values for MSE, width of the 95% confidence interval, and relative error of lean and lipid mass. Although my results did not preclude one approach as being superior to the other, they did agree with the opinion of Scott et al. (1991), who argued that TOBEC is highly reproducible and can appropriately be used as the independent variable. However, it was also clear that if lipid mass is the variable of interest, the debate over which approach is superior becomes a mute point because two-stage models have not predicted lipid mass with an acceptable level of accuracy in my study or in previous studies.

Lyons and Haig (1995) presented results of models that included TOBEC values or TOBEC values transformed for body geometry. Their study presented intraspecific models for each of three species of shorebirds and an interspecific model, which included all three species in one predictive equation. The use of the transformation produced lower 95% prediction intervals in all but one intraspecific model and for the interspecific model, however the improvements were minimal. The transformation also produced lower percent error in predicted lean mass. The improvement was greatest for the interspecific model, in which percent error was reduced from 8.78 ± 1.41 to 3.46 ± 0.59% by the transformed TOBEC value. In my study, the transformed TOBEC values also provided a marginal improvement in prediction of fat-free mass in the two-stage models, however they did not improve direct models.

Voltura and Wunder (1998) found that the use of a direct model did not improve the relative error of predicting lipid mass in captive and wild prairie voles over that of a two-stage model. My study conflicted with their findings in that I found a large difference in the relative error of predicted lipid mass in eastern woodrats between the two-stage model and the direct model (104.39% vs. 24.84%), even though the relationship between lipid mass regressed on TOBEC value and body mass was not as strong in my study ($\underline{R}^2 = 0.42$ vs. $\underline{R}^2 = 0.94$). Voltura and Wunder (1998) stated that because prairie voles remain relatively lean throughout the year, the error associated with predicting lipid mass in their models represented 50-100% of the total amount of lipid that a prairie vole would carry at any time. Therefore, they did not recommend the use of TOBEC for relatively lean mammals, such as prairie voles. However, the amount of lipid mass that a mammal carries would be more important in the accuracy of TOBEC than how lean the mammal is because it is total lipid mass that will determine the TOBEC reading, not the proportion of lipid mass to body mass. Although eastern woodrats were leaner than prairie voles (3.74% vs. 13.65%), they carried 50% greater lipid mass than prairie voles (9.64g vs. 6.25g). The absolute error associated with the predicted lipid mass in my study represented 22% of the mean lipid mass of eastern woodrats included in the study. Although that value is higher than would be desired for a predictive model, the use of TOBEC to predict lipid mass in eastern woodrats remains the most accurate non-invasive method currently available. The use of TOBEC in body composition studies should include an analysis of associated errors in predicted lean and lipid mass. I suggest that the eastern woodrat may fall at the lower end of a range of body sizes of wild mammals in which TOBEC can potentially predict lipid mass within an acceptable level of accuracy (e.g. ground squirrels may be the most appropriate size). Future TOBEC studies with mammals need to determine what range of body sizes can accurately be used with TOBEC and what level of accuracy is acceptable for predicting lipid mass in TOBEC studies.

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CHAPTER FIVE
CONCLUSIONS

Eastern woodrats (Neotoma floridana) showed definite seasonal changes in hematology, body composition, and food caches. These changes reflected the expected energetic demands of each season. Winter is an energetically demanding season for a small mammal due to the increased energy demands of thermoregulation. Eastern woodrats showed a very clear pattern in hematology related to such winter energy demands. The results of chapter two showed that patterns of hematological changes in eastern woodrats were related to seasonal changes in ambient temperature, which reflected seasonal differences in metabolic activity. For packed cell volume (PCV) and red blood cell (RBC) counts, highest values occurred in the winter when metabolism for thermoregulation should be highest and the lowest values occurred in the summer. Fall and spring represented intermediate levels of PCV and RBC counts. Although Hb levels were expected to be at their highest levels in the winter, in order to meet needs for increased oxygen capacity, they were at their lowest and showed evidence of an iron-deficiency anemia. Low Hb levels likely reflect the effects of a nutritional stress on winter eastern woodrats, however they may also represent the effects of increased consumption of phenolic compounds. The low winter Hb levels shifted the peak levels into the spring, which coincided with the beginning of the breeding season. Although sex was not determined to be playing a major role in my study, it is likely a factor influencing hematological values during the breeding season. The thermal benefits of the eastern woodrat house were not enough of a barrier to temperature fluctuations to eliminate the need for physiological adjustments and eastern woodrats were determined to exhibit seasonal changes in hematological values similar to those found in other small mammal species.

The results detailed in chapter three showed that eastern woodrats demonstrated a definite pattern in the relationship of energy stored as internal energy (body fat) and external energy (food cache). High external stores relative to internal stores and apparent conservation of lipids characterized winter. Spring was characterized by a surplus of internal energy stores. Serum chemistry analysis showed plasma lipid levels were lowest in the winter, which agreed with the pattern of decreased lipid mobilization in the winter. Plasma protein concentrations were constant throughout the seasons studied. Such a strategy would have apparent benefits in available energy for reproduction in the spring, when internal energy stores were highest and external energy stores were lowest. In such a case, the larder hoarder benefits from the cost of obtaining and defending its larder by a surplus of internal energy for reproduction in the spring, thereby maximizing the efficiency of converting resources into offspring.

Chapter four examined the use of total body electrical conductivity (TOBEC) for predicting lean and lipid mass in eastern woodrats. Two-stage models were accurate at estimating lean mass, however they estimated lipid mass poorly (relative errors in predicted lipid mass of over 100%). The direct models, in which lipid mass was predicted directly from body mass and TOBEC values, were more accurate at predicting lipid mass in eastern woodrats. The best direct model predicted lipid mass with a relative error of 24.84%. There was not a large difference in the performance of two-stage models that used TOBEC value as the independent or dependent variable. Also, the use of a TOBEC value transformed for body geometry only marginally improved the performance of two-stage models and did not improve direct models. TOBEC predictive equations of lipid mass were determined to be the most accurate non-invasive method of predicting lipid mass in eastern woodrats currently available.

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