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BODY COMPOSITION DYNAMICS OF A SMALL HERBIVORE, THE MEADOW VOLE, (*MICROTUS PENNSYLVANICUS*): A FIELD AND LABORATORY PERSPECTIVE

Submitted by

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Graduate Degree Program in Ecology

In partial fulfillment of the requirements

for the degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 1998

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY EDWARD T. UNANGST, JR. ENTITLED BODY COMPOSITION DYNAMICS OF A SMALL HERBIVORE, THE MEADOW VOLE, (*MICROTUS PENNSYLVANICUS*): A FIELD AND LABORATORY PERSPECTIVE BE ACCEPTED AS FULFILLING PART OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Advisor

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ABSTRACT OF DISSERTATION

BODY COMPOSITION DYNAMICS OF A SMALL HERBIVORE,
THE MEADOW VOLE, *MICROTUS PENNSYLVANICUS*:
A FIELD AND LABORATORY PERSPECTIVE

In the field, meadow voles are relatively lean year-round, maintaining between 2 to 3 g of lipid. Percent body fat of individuals varied seasonally due primarily to losses in body mass of adult voles and reduction in growth rates by subadults during winter. When brought into the laboratory, voles significantly increased body mass due to large gains in lipid mass and small decreases in fat-free mass, regardless of season of capture or diet quality. Within three weeks, voles increased body fat from 5 to 25 % whether eating lab chow (5 % dietary fat) or rabbit chow (1.5 % dietary fat). If dietary fat was increased from 5 to 25 %, voles increased body fat to 30 % within three weeks. When dietary fat was reduced from 25 to 5 %, voles lost 8.69 g of body mass and 6.41 g of body fat after three weeks. Regardless of diet, voles regulated body mass and body composition at levels which correspond to dietary quality and abundance. With running wheels, voles ran 1.17 kilometers per day but did not reduce lipid deposition. The relationship between the level of activity and change in all body composition parameters was not correlative suggesting no activity effect on vole body composition. With supplemental high-fat food in the field, resident voles increased lipid mass to levels atypical of field animals but less than those observed in the lab.

When held in the lab, field voles increased lipid levels five fold within six weeks, yet plateau and maintain a body composition directly with diet quality and abundance.

Potential causes for laboratory fattening include high quality, highly digestible diet, stable environmental conditions, and decreased activity. We confirmed the effects of unnatural diets on increased body fat but cannot substantiate activity effects on decreased body fat. Nevertheless, the body composition of a field animal held under laboratory conditions is an animal with a very different body composition in a relatively short time period. We caution researchers to account for effect in their research and when extrapolating results gathered from lab-reared and lab-held animals to their field counterparts.

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Experiments conducted as part of this research were approved by Colorado State University Animal Care and Use. Components of the research conducted exclusively at the United States Air Force Academy were also approved by their Animal Care and Use committee.

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Chapter 1

Introduction and literature review

The study species

The meadow vole (*Microtus pennsylvanicus*) belongs to the order Rodentia, family Muridae, subfamily Arvicolinae (voles, lemmings, and muskrats) (Carleton and Musser 1984). As a group, voles and lemmings are still commonly referred to as microtine rodents, an archaic classification, and will be used exclusively in this manuscript. Microtine rodents originated in Asia and migrated to North America over the Bering Land Bridge during the Pleistocene (Repenning 1980). The first migration occurred in the early Pleistocene, approximately 1.8 - 2.0 million years ago, with the more modern voles with rootless molars arriving in later migrations (1.2 million years ago, Repenning 1980; Zakrzewski 1985).

The most advanced of North American microtine genera, *Microtus* currently has 23 species in the New World (Anderson 1985). In comparison, there are currently 48 species of Old World voles (Honacki et al. 1982) which is most likely due to a much larger area in the Palearctic (fourteen million square kilometers) compared to six million square kilometers for the Nearctic. Only one species, *Microtus oeconemus*, occurs on both sides of the Bering Strait, and thus displays a Holarctic distribution.

The range for voles of the genus *Microtus* extends from the tundra biome in Alaska and Canada to the cloudforest in the montane highlands of Guatemala (Hoffman

and Koeppl 1985). Intermediate to these locale extremes, voles also occupy montane pine-oak and subtropical deciduous habitats. Within these regions, voles occupy all but the most xeric habitats. Within North America, the meadow vole (*M. pennsylvanicus*) has the widest distribution and prefers grassy meadow areas within coniferous forests (Getz 1985). In the United States, meadow voles are present in the Appalachian and Rocky Mountains, as well as the eastern deciduous forests and the grasslands of the northern plains. In the Pacific coastal taiga, meadow voles are sympatric with *M. townsendii*. In locations where meadow voles inhabit similar overlapping habitat with other vole species such as *M. ochrogaster* or *M. montanus*, they exist allopatrically.

The history of *M. pennsylvanicus* dates back to late mid-pleistocene, about 500,000 years ago. Its lineage may have given rise to several other lineages adapted to taiga meadows, such as *M. montanus* and *M. canicaudus* in the Rocky Mountains and the Sierras and Cascades. Within the United States, fossils of meadow voles have been found in over half of the states, as well as in all Canadian provinces (Zakrzewski 1985).

Microtine rodents share a common body plan and are described as "robust animals with short legs and tails, blunt muzzles, diminutive pinnae, and small eyes" (Carleton 1985). Consistent with most voles, data on meadow voles indicate that they are primarily promiscuous (Wolff 1985). Reproduction is typically seasonal, with primary activity occurring from late spring to early fall and occasional breeding in winter (Wolff 1985). All females undergo a post-partum estrus, which results in a complete overlap of pregnancy and lactation (Hasler 1975). Therefore, most breeding females are usually lactating throughout the reproductive season (Keller 1985).

Among the world's smallest mammalian vegetation herbivores, meadow voles forage primarily on the vegetative parts of grasses and sedges, a diet which is of comparatively poor quality (Batzli 1985; Batzli and Cole 1979; Bergeron and Jodoin 1987; Goldberg et al. 1980). Meadow voles possess a number of adaptations for a fibrous diet. These adaptations include rootless, high-crowned molars for grinding food and a large cecum for the digestion of fiber. As a generalist herbivore, meadow voles are able to inhabit and perpetuate in many parts of North America. Their distribution encompasses a wide range of habitats and climatic conditions with variability in ambient temperature, food quality and food abundance. To cope with such a wide variation in environmental conditions, meadow voles possess an adaptive suite of traits, both behavioral and physiological.

No microtine rodent shows any evidence of either daily or extended torpor. Meadow voles possess specialized brown fat adipose tissue which can generate additional heat through non-shivering thermogenesis (NST). The brown fat can vary both in amount and cellular content, dependent upon season, with the highest NST capacity in the winter months (Didow and Hayward 1969). In addition, microtines will increase fur length and have greater fur density in winter. Finally, microtines increase food intake when exposed to cold temperatures (Bergeron and Jodoin 1987; Brochu et al. 1988; Castle and Wunder 1995; Hammond and Wunder 1991; Voltura 1996).

Meadow voles will also increase food intake in response to poor quality, high fiber food. With increasing fiber content, voles increase food intake (Castle and Wunder 1995; Hammond and Wunder 1991; Keys and VanSoest 1970; Voltura 1997). The increased

food intake is accomplished in part by an increase in the size and volume of the gastrointestinal tract (Gross et al. 1985; Dertling and Bogue 1993; Dertling and Nokes 1995; Hammond and Wunder 1995; Voltura 1997).

Body composition dynamics and lipogenesis

To ensure survival, small mammals have adaptive strategies based on either energy storing or energy sparing. Hibernation (energy sparing) is often combined with physiologic changes such as increased deposition of body fat (energy storing) in species like marmots (*Marmota sp.*) and ground squirrels (*Spermophilus sp.*). Variations to the energy sparing strategy also include the use of daily torpor as demonstrated in deer mice (*Peromyscus sp.*). Species such as pika (*Ochonta princeps*), woodrats (*Neotoma sp.*) and ground squirrels store energy (food caching) to be consumed during periods of food shortages or increased energy need.

During winter, microtines may be faced with a precarious energy balance. Their relatively small body size (less than 80 g) results in a high surface area to volume ratio, which is conducive to high heat loss under colder conditions (Kleiber 1947). When exposed to colder ambient temperatures, increases in metabolic demands result and must be met by either behavioral and/or physiologic strategies (Wunder 1984, 1985). In voles and lemmings, a capacity for torpor has not been demonstrated. To combat heat loss, voles and lemmings demonstrate behaviors such as reduced activity, nest building, and huddling (Grodzinski and Wunder 1975; Madison 1984, 1985; Webster and Brooks 1981; Wolff 1985). Communal nesting and storing food in the vicinity of the nest occur in several species of *Microtus*. Food caching by meadow voles has been reported in winter

(Gates and Gates 1980; Riewe 1973) and may occur in other species of *Microtus*. In addition, increases in gut mass and volume allow increased food intake and greater digestion through longer retention and passage rates (Gross et al. 1985; Dertling and Bogues 1993; Dertling and Nokes 1995; Hammond and Wunder 1995; Voltura 1997).

The use of energy reserves (body fat) can be vital to the overwinter survival of animals that remain active all year in temperate environments and may be a useful indicator of physiological condition. Adipose tissue can serve as a reservoir for the storage of energy and as a thermal insulator. However, the need for energy reserves must be balanced by the costs of fat storage. Heavy stores of body fat have advantages as well as disadvantages. Body fat is an efficient means of storing energy. Not only does fat supply about twice as many calories as an equivalent mass of carbohydrates, but fat can also be stored without hydration, which is a further saving in both weight and bulk as compared to lean tissue. These characteristics make body fat a suitable energy store for physiological, reproductive, and behavioral events. In contrast, the disadvantages of heavy fat stores include decreased mechanical performance through locomotion impedance, and potential increased predation risk (Pond 1978, 1981). However, seasonal fluctuations in body fat stores appear to be evolutionary adaptations that make heavy fat stores available when they can have an adaptive role and eliminate them at other times.

The amount of body fat present at any one time is the net effect of deposition and usage. Fat deposited in adipose tissue and other organs, such as the liver, may be derived by either dietary fat or *de novo* synthesis. The nutritional state of the animal strongly influences fat synthesis (lipogenesis). Well fed individuals which eat a diet high in

carbohydrates have high rates of lipogenesis. In most mammals and other vertebrates, lipogenesis occurs primarily in the liver with primary storage in white adipose tissue as triacylglycerols. Because of requirements for specific coenzymes and substrates provided by carbohydrate metabolism, lipogenesis cannot occur independent of carbohydrate metabolism with glucose serving as the substrate for fat synthesis. Diets high in polyunsaturated fats simplify the conversion to body fat because of the presence of available fatty acids. In ruminants (and most likely herbivorous rodents), acetate is the primary substrate for lipogenesis.

In the field, small mammals (excluding hibernators) are typically very lean, with fat content of 3 to 8 % of total body mass (Table 1.1). There exists varying interpretations about seasonal effects of body composition. Many studies suggest that body fat depends on a complex interaction of numerous factors including age, sex, season, and nutritional status. The relative contribution of these factors is difficult, if not impossible, to discern in the field and has led to laboratory manipulations. In studies where seasonal change in body composition is reported, percent body fat is usually lower in summer than in winter (Anderson and Rauch 1984; Batzli & Esseks 1992; Cengel et al. 1978; Gyug and Millar 1989; Rock and Williams 1979; Schreiber and Johnson 1975; Voltura 1997). This is due in part to reproductive females maintaining very low fat levels in summer (Gyug and Millar 1980; Lochmiller et al. 1983; Millar 1981; Voltura 1997) as well as seasonal differences in growth rates and maturation (Barbehenn 1955; Fuller et al. 1969; Brown 1973; Iverson and Turner 1974; Peterborg 1978). However, seasonal change in body fat is not widespread among many non-hibernating mammals. No seasonal trends in lipid levels were

found in deer mice (*Peromyscus maniculatus*) (Morton and Lewis 1980; Schreiber and Johnson 1975), or prairie voles (*Microtus ochrogaster*) (Baker 1971; Fleharty et al. 1974; Morton and Lewis 1980).

In contrast to the lean body composition found in the field, small mammals have the ability to deposit substantial amounts of body fat when reared in the lab or removed from the field and kept in the lab. When small mammals are removed from the field and held under laboratory conditions (Batzli and Esseks 1992; Ferns and Adams 1974; Sawicka-Kapusta 1970; Voltura 1996, 1997) or reared within the lab (Donald et al. 1980; Holleman and Dieterich 1978; Sawicka-Kapusta 1970, 1974; Voltura and Wunder 1998), they increase body fat within weeks to levels atypical of field observations. In these studies where animals are kept in the laboratory, body fat often exceeds 10 % of body mass within 30 days. The potential causes for such body composition change may be the effects of confinement (which reduces activity), unnatural, highly digestible diets, stable environmental conditions, or any combinations thereof. In addition, laboratory studies have shown that both body mass and growth rates are affected by the interactions of photoperiod, temperature, and diet quality for collared lemmings (*Dicrostonyx groenlandicus*) (Mallory et al. 1981; Nagy 1983; Nagy and Negus 1983; Nagy et al. 1994), montane voles (Horton 1984a, 1984b; Pinter 1968; Peterborg 1978; Vaughan et a. 1973) and meadow voles (Dark and Zucker 1983; Dark et al. 1983; Pistole and Cranford 1982). In collared lemmings (Nagy and Negus 1983), body mass increased under conditions simulating winter (short day photoperiod, cold temperature, and high food quality). In montane and meadow voles, the opposite effect on mass and growth occurs

with winter, as body mass decreases and growth rates slow. In these studies, the effects on body composition were addressed with invasive chemical extraction methods, whereas the technology for obtaining repeated measures of body composition in small mammals has only recently become available.

Research questions

The objectives of my research were to examine meadow vole body composition dynamics in both the field and laboratory and to determine seasonal effects and potential factors influencing changes in body composition. If meadow voles are lean in the field, is it the effect of diet quality? Will meadow voles change body composition when held in the lab and why does this occur?

The meadow vole is a small, non-hibernating, herbivorous rodent and does not cache substantial amounts of food, nor go torpid in winter months. Fat reserves may provide small mammals with necessary energy during periods of energy crises. The amount of body fat in the field may be the result of food quality, ambient temperature stresses, activity, or any combinations thereof.

In the chapters that follow, I will report on field studies examining body composition dynamics as well as changes in body composition when voles are removed from the wild and held in the laboratory. I will assess the effects of diet quality and activity on body composition and report on diet preference in relation to dietary fat. I will

also report on the utility and application of a non-invasive device (EM-SCAN®) used to estimate body composition in the lab and under livetrap conditions.

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Table 1.1. Percent body fat of wild, non-hibernating small mammals.

Species	Fat Content % Total Body Mass	Reference
Meadow vole (*Microtus pennsylvanicus*)	3 - 7	1
	3 - 8	9
Pine vole (*Microtus pinetorum*)	5 - 7	5
Prairie vole (*Microtus ochrogaster*)	4 - 5	6
	3 - 5	8
Red-backed voles (*Clethrionomys gapperi*)	3 - 5	1
Brown lemming (*Lemmus sibiricus*)	3 - 5	2
Deer mouse (*Peromyscus maniculatus*)	3 - 5	7
	5 - 7	3, 6
White-footed mouse (*Peromyscus leucopus*)	5 - 8	4
Harvest Mouse (*Reithrodontomys megalotis*)	3 - 5	7
	6 - 7	3
Grasshopper mouse (*Onychomys leucogaster*)	4 - 6	7

1. Anderson and Rauch 1984
2. Batzli and Esseks 1992
3. Fleharty et al. 1973
4. Lynch 1973
5. Lochmiller et al. 1983 (data provided as % dry mass and converted to % wet mass)
6. Morton and Lewis 1980
7. Schreiber and Johnson 1975
8. Voltura 1997
9. This study

Chapter 2

Use of the EM-SCAN® SA-2 to measure body composition of the meadow vole (*Microtus pennsylvanicus*)

INTRODUCTION

Most studies of body composition in small mammals have used chemical extraction of whole body homogenates as their principal method. Such methods, although accurate, have one serious drawback: the need to euthanize the subject. Thus, animals must be removed from a population or study and repeated measures cannot be made. However, the need to measure body composition in live animals and to perform repeated measures on individuals exists. Such determination can be made noninvasively through measurements of total body electrical conductivity, commonly referred to as TOBEC. Initial TOBEC technology was developed and applied in the early 1980s to determine body composition in domestic and laboratory animals (Bracco et al. 1983; Harrison & Van Itallie 1982; Horswill et al. 1989; Keim et al. 1988) and numerous devices using TOBEC or similar technology to estimate body composition are currently available. One such procedure uses a device known as the EM-SCAN® Small Animal Body Composition Analyzer (EM-SCAN Inc., Springfield IL). The application of EM-SCAN® in ecological studies was first introduced by Walsberg in 1988. Subsequent studies on wild species have become more widespread using birds (Castro et al. 1990; Morton et al. 1991; Osborne et al. 1996; Roby 1991; Scott et al. 1991, 1996; Skagen and Knopf 1993), small mammals (Bachman 1994; Voltura 1997), and fish (Fischer et al. 1996). In addition, EM-

SCAN® has been used to investigate body water content (Cunningham et al. 1986; Cochran et al. 1989; Guggenbuhl 1996) and metabolism (Presta et al. 1983; Scott et al. 1996) in relation to body composition.

The principles upon which the EM-SCAN® Small Animal Composition Analyzer operates are detailed by numerous researchers (Castro et al. 1990; Cochran et al. 1989; Fischer et al. 1996; Walsberg 1988). Generally, body composition estimates are based upon the concept that the sum of fat-free mass and lipid mass equals total body mass. To determine body composition with EM-SCAN®, a common approach is to generate a linear regression (simple or multiple) which estimates fat-free mass from the EM-SCAN® reading. Known as the two stage model, the lipid mass estimate is then calculated by subtracting the fat-free mass estimate from total body mass. With this approach, the regression of fat-free mass to EM-SCAN® readings often results in high R^2 values (Castro et al. 1990; Osborne et al. 1996; Scott et al. 1991, 1996; Voltura 1996; Walsberg 1988). Morton et al. (1991) contend that these high R^2 values misrepresent the accuracy with which lipid mass can be estimated. Although the absolute error associated with estimating fat-free mass is the same as the absolute error associated with calculated lipid mass, the relative error is much higher for lipid mass, because lipids are typically a much smaller proportion of total body mass (Morton et al. 1991). As a result, Voltura (1996) used multiple regression techniques to directly predict lipid mass. Thus, a reduction in error for lipid mass estimates resulted, since lipid mass was estimated directly by the regression.

Other aspects of the animal's physiologic state may affect EM-SCAN® estimates. Walsberg (1988) noted the need to ensure that animals are not dehydrated when EM-SCAN ® estimates are performed, since EM-SCAN® methods are based on a fat-free mass being approximately 20 times more hydrated than lipids. As a result, changes in hydration will differentially affect estimates of fat-free and lipid with regard to total body mass. In addition, Walsberg (1988) alleged that deviations in body temperature of more than 5° C can also affect EM-SCAN® estimates. Lastly, the contents of the gastrointestinal tract, which are read by EM-SCAN® as fat-free mass, (Bachman 1994; Voltura 1996) can also lead to variation in estimates.

Although generic calibration equations for several species groups, such as rodents, are provided by the EM-SCAN ® manufacturer, they recommend that researchers derive species-specific calibration equations to increase estimate accuracy. The generic rodent equation was derived using laboratory rats, which may not be similar morphologically or physiologically to a specific research species. As a result, this equation may lack the accuracy necessary for many studies. In a study of prairie voles, Voltura and Wunder (1998) concluded that the EM-SCAN® rodent model does not work with voles. However, it is possible that species-specific equations may not be necessary for species which share similar morphology.

To address these issues, we derived a species-specific calibration equation for meadow voles (*Microtus pennsylvanicus*) using linear and multiple regression techniques. To test both the need to derive a species-specific equation and the use of a "general morph" equation, we compared body composition estimates from our equation to two

other equations, the generic rodent equation provided by EM-SCAN® and a multiple regression equation derived for prairie voles (*M. ochrogaster*) by Voltura (1996). Since prairie voles are very similar in size and shape to meadow voles, they would be an ideal species to test our "general morph" hypothesis.

MATERIALS AND METHODS

Twenty-seven adult meadow voles were used to establish an EM-SCAN® Model SA-2 calibration curve for the species. All voles were trapped at the United States Air Force Academy, El Paso County, Colorado on 14 August 1996. After capture, voles were housed in individual cages (28 x 18 x 13 cm) maintained on a photoperiod of 16L:8D at 23° C and fed lab chow (Lab Diet 5001, PMI Feeds) and water *ad lib.* Body composition estimates were made the following day on 16 of the 27 voles using the EM-SCAN® SA-2 Small Animal Body Composition Analyzer (EM-SCAN Inc., Springfield IL); those animals were then euthanized and frozen for later analysis of body composition using standard techniques. Since voles maintained in the lab have more body fat than voles from the wild, the remaining 11 voles were maintained in our laboratory, under conditions noted above, for an additional month to increase lipid mass.

Since any change in body position of the measured subject within the EM-SCAN® device will affect the reading, voles were anesthetized. I first used methoxyfluorane (Metophane®, Mundelein, IL) and then interperitoneally injected a mixture of Ketamine (15mg/kg) (Fort Dodge Laboratories, Inc., Fort Dodge, IO) and Xylazine (3mg/kg)

(Vedco, Inc., St. Joseph, MO). Voles were then weighed to the nearest 0.01 g (Ohaus E400D) and body length without tail was measured (nearest 1.0 mm) by stretching animals out along a 250 cm ruler. Body length without tail was used to center the vole both within the EM-SCAN® SA-2 chamber and on the insertion platform. Seven readings were taken on each animal, the highest and lowest values were omitted, and the remaining five values were averaged as in Voltura and Wunder (1998). Since EM-SCAN readings are affected by body geometry, an EM-SCAN index was calculated that included the average EM-SCAN® reading and the body length (without tail) (Fiorotto et al. 1987; Voltura and Wunder 1998). The formula for the index is given below:

$$\text{EM-SCAN index} = (\text{average EM-SCAN reading} \times \text{body length})^{0.5} \quad (1)$$

After the final EM-SCAN® reading was taken, each vole was immediately sacrificed by methoxyfluorane overdose and the carcasses frozen at -20° C. To prepare the carcasses for soxhlet chemical extraction of lipids, the fur was shaved to facilitate grinding and the gastrointestinal tract removed by dissection. The carcass, fur, and gastrointestinal tract with contents were then dried to constant mass at 60° C using a forced air convection drier. Total body water was calculated as the mass differences between initial and constant mass. Once dried, the carcass and gastrointestinal tract were ground in a coffee grinder to ensure homogeneity and the entire contents used in lipid extraction.

Chemical extraction of lipids was performed by a contracted laboratory at the University of Western Ontario, London, Ontario, Canada using a modified soxhlet

procedure. The general procedure was to dry cellulose thimbles to a constant weight (thimble weight) in a drying oven at approximately 80° C (Kerr et al. 1982) and then fill a thimble with the entire dry, ground vole homogenate for one specimen. If the tissue from one vole would not fit into one thimble, it was roughly divided between two thimbles. Data for voles divided between two thimbles were added together to perform calculations for each vole. Then , thimbles and samples were dried to constant weight (thimble + sample dry weight) at 80° C and the fat extracted using petroleum ether (Dobush et al. 1985) in a modified soxhlet apparatus (modified to extract the fat from 20 samples at once rather than 1 at a time). Following lipid extraction, thimbles and lean samples were again dried to constant mass at 80° C (thimble + lean dry mass).

Calculation of lipid mass is as follows:

$$\text{Lipid mass} = \frac{(\text{thimble + sample mass (g)}) - (\text{thimble + lean mass (g)})}{(\text{thimble + sample mass (g)}) - (\text{thimble mass (g)})} \times \text{carcass dry mass (g)} \quad (2)$$

$$\text{Fat-free mass (g)} = \text{carcass dry mass (g)} - \text{lipid mass (g)} \quad (3)$$

Using the actual body composition values from the chemical extraction, calibration equations to estimate body composition were derived using both simple linear regression (Walsberg 1988; Castro et al. 1990; Voltura 1996) and multiple regression techniques (Voltura 1996). The simple linear regression model used the EM-SCAN index (1) as the dependent variable and fat-free mass as the independent variable (measured by extraction) (Morton et al. 1991, Voltura 1996). In contrast, the multiple regression model used lipid mass as the dependent variable as in Voltura (1996). Potential independent variables tested in the multiple regression model included the EM-SCAN® index (1), EM-SCAN®

reading alone, body length alone, and body mass. Other standard mammalogy measurements for size (e.g., right hind foot and ear length) were not considered since Voltura (1996) found they did not add to the precision of estimates in prairie voles. An adjusted R^2 model selection technique (SAS Institute, Inc., 1989) was used with the 10 best models evaluated and parsimoniously selected for best fit.

To evaluate the need for species-specific models, we compared the average error in estimates of lipid mass and percent body fat resulting from the multiple regression equations for meadow voles (this study), prairie voles (Voltura and Wunder 1998), and generic rodent (EM-SCAN® SA-2 manufacturer).

The *Microtus ochrogaster* (prairie voles) equation (Voltura 1996) is:

$$\text{Lipid mass} = 2.122 + (0.861 \times \text{body mass}) - (0.528 \times \text{EM-SCAN index}) \quad (4)$$

Fat-free mass = total body mass - lipid mass

where EM-SCAN index = (average EM-SCAN reading x body length)$^{0.5}$.

The generic rodent equation (EM-SCAN® SA-2 manufacturer) is:

$$\text{Fat-free mass} = 30.84 + (0.396 \times E) - (4.85 \times 10^{-5} \times E^2) \quad (5)$$

Lipid mass = total body mass - fat-free mass

where E = the average EM-SCAN reading.

Using the EM-SCAN® reading and relevant morphologic measurements (total body mass, body length) of the entire meadow vole data set (n = 27 voles), we calculated the average error in estimates of lipid mass and percent body fat resulting from each equation. We then compared the average error in estimates for each equation to

determine if reductions in the average error of estimate were evident. The initial comparison used the complete data set since it encompassed the widest range of variation in body composition. Two additional comparisons using two subsets of the complete data were also made. One subset consisted of 16 wild voles with relatively low body fat and referred to as the "lean" data subset. The other subset, referred to as the "fat" data subset, contained 11 voles which were captured in the field, but maintained in the lab for one month to increase lipid levels. The use of data subsets allowed us to investigate estimate accuracy under narrower body composition ranges. We did that because the body fat level of voles used by Voltura and Wunder (1998) nearly approximates our "fat" data set. We could not perform statistical comparisons of estimate errors between equations because of violations in the assumption of independence within the data set. The average error in estimates was derived as follows:

Average error, lipid mass = ABS (actual lipid (g) - estimated lipid (g)) (6)

Average error, % body fat = ABS (actual % lipid - estimated % lipid) (7)

where ABS is the mean of the absolute value.

To determine the average error in estimates for our multiple regression equation, we could not use the same procedure. Since our calibration equation was initially derived from the complete meadow vole data set, the resulting regression was a "best fit" which attempts to reduce the residual in estimates. As a result, the error in estimates would be biased and artificially low. Therefore, cross validation was used for our equation to determine the average error in estimates (Conway et al. 1994; Skagen and Knopf 1993;

Voltura and Wunder 1998). In this procedure, the average error of estimates was predicted using the data set with one vole removed. This process was repeated, removing a different vole each time, until all voles were accounted. Then the average error was calculated as the mean of the average error in all runs. The net result of this procedure is a slight increase in average error for our equation.

RESULTS

The complete data set used in the calibration equation derivation for *M. pennsylvanicus* contained 16 wild-caught voles and 11 wild-caught voles maintained under laboratory conditions for one month. Body composition data for the complete, "lean" and "fat" data subsets, are presented in Table 2.1. For the complete data set, percent total body water was 63.28 ± 1.47 % (range 46.64 to 71.76 %). Mean total body mass was 47.07 ± 1.91 g (range 26.94 to 65.41 g). Fat-free mass averaged 41.15 ± 1.97 g (range 21.65 to 63.08 g) and lipid mass was 5.92 ± 0.95 g (range 1.55 to 20.79 g). Percent body fat, expressed as a percent of total body mass, was 12.75 ± 1.91 % (range 3.52 to 33.30 %).

Using the complete data set, the *M. pennsylvanicus* multiple regression equation explained 85.9 % of the variation in lipid mass, with an average error in lipid mass estimates of 1.55 ± 0.18 g. This equates to an average error in percent body fat estimates of 3.56 ± 0.50 % (Table 2.2). The two-stage linear model for *M. pennsylvanicus,* with the EM-SCAN® index as the dependent variable, explained 97.1 % of the variation in fat-free

mass, with an average error for estimating lipid mass of 1.60 ± 0.18 g. The average error in percent body fat estimates was 3.68 ± 0.50 % (Table 2.2).

The "lean" subset body composition is summarized as follows: total body mass was 47.91 ± 2.61 g (range 26.94 to 65.41 g). Fat-free mass averaged 45.07 ± 2.53 g (range 24.15 to 63.08 g) and lipid mass was 2.84 ± 0.27 g (range 1.55 to 5.02 g). Percent body fat was 6.07 ± 2.53 % (range 3.52 to 10.36) (Table 2.1). Using this subset, the *M. pennsylvanicus* multiple regression model had an average error in lipid mass estimates of 1.44 ± 0.26 g, and an average error in percent body fat estimates of 3.36 ± 0.62 % (Table 2.2). The *M. pennsylvanicus* two stage model, with the EM-SCAN® index as the dependent variable, had an average error in lipid mass estimates of 1.52 ± 0.27 g. This equates to an average error in body fat estimates mass of 3.24 ± 0.66 % (Table 2.2). For the "lean" subset, percent total body water was 68.37 ± 0.45 % (range 64.14 to 71.76 %).

In the "fat" subset, total body mass averaged 45.85 ± 2.83 g (range 29.58 to 66.61 g). Fat-free mass was 35.44 ± 2.32 g (range 21.65 to 47.89 g), with a lipid mass average of 10.41 ± 1.47 g (range 3.37 to 20.79 g). Percent body fat averaged 22.47 ± 2.60 % (range 9.93 to 34.30) (Table 2.1). The *M. pennsylvanicus* multiple regression equation had an average error in lipid mass estimates of 1.70 ± 0.25 g and a percent body fat average error in estimates of 4.16 ± 0.86 % using the "fat" subset (Table 2.2). The linear model for *M. pennsylvanicus,* with the EM-SCAN® index as the dependent variable, had a lipid mass average error in estimates 1.71 ± 0.23 g. This equates to an average error in percent body fat estimates of 4.03 ± 0.78 % (Table 2.2). Reflecting the increase in body fat, percent total body water was 55.89 ± 2.05 % (range 46.64 to 65.87 %).

The prairie voles used by Voltura and Wunder (1998) to derive their calibration equation had much higher lipid mass and percent body fat level than the complete data set used in our study. However, the body composition of the prairie voles were very similar to the "fat" data subset (Table 2.3). Voltura and Wunder (1996) held all prairie voles used in the calibration equation in the lab for approximately two months before body composition was measured. For the prairie voles, total body mass was 47.0 ± 1.7 g (range 33.8 to 58.5 g). Fat-free mass averaged 37.4 ± 1.4 g (range 26.7 to 47.0 g) with a lipid mass average of 9.6 ± 0.8 g (range 5.3 to 13.8 g). Percent body fat was 20.4 ± 1.4 % (range 12.2 to 30.9) (Table 2.3). For *M. ochrogaster,* the multiple regression equation of Voltura and Wunder (1998) explained 79.7 % of the variation in lipid mass, with an average error in lipid mass estimates of 1.02 ± 0.20 g. However, when applying this model to our data (complete and two data subsets for *M. pennsylvanicus*), estimation error increased in all cases as presented in Table 2.4. The lowest error in lipid mass estimates (2.25 ± 0.54 g) was associated with the "fat" subset. This was expected since the body composition of voles used to derive the calibration equation was very similar to the body composition in the "fat" subset.

Body composition data of laboratory rats used by the manufacturer to derive the generic rodent model were not available. However, since the average size and shape of laboratory rats is quite different than meadow voles, we did not expect low average error in estimates. As expected, the average error in estimates was quite high for all data sets as presented in Table 2.5. The best performance resulted in an average error in lipid mass estimates of 4.04 ± 0.81 g and a percent body fat estimate average error of 9.76 ± 2.71%.

Such precision would be unacceptable if used to estimate the body composition of lean animals, a common condition in many species of small mammals in the wild.

DISCUSSION

This study confirms the use of EM-SCAN® as an effective alternative to traditional invasive methods for determining body composition of voles. The EM-SCAN® reading provided by the EM-SCAN® SA-2 device, combined with pertinent morphological measurements, resulted in accurate estimates of both fat-free mass and lipid mass comparable to previous studies (Bachman 1994; Baer et al. 1991; Bell et al. 1994; Castro et al. 1990; Gosselin & Cabanac 1996; Horswill et al. 1989; Keim et al. 1988; Skagen and Knopf 1993; Stenger 1995; Voltura 1996). Our results also suggest that deriving a species-specific calibration equation will minimize error in estimates under most circumstances. However, under certain conditions, we feel that a more general "morph" equation may meet researcher needs and eliminate the need to derive a species-specific equation.

To be a useful tool for estimating body composition, both in the laboratory and the field, the device must be as simple as possible, yet maintain a high degree of estimation accuracy. With proper precautions and a consistent protocol, the EM-SCAN® can achieve such results. The two-stage linear regression model, which correlates fat-free mass to the EM-SCAN® index, is very simple and can result in very high accuracy in estimating fat-free mass ($R^2 = 0.971$) (this study) (Figure 2.1). However, since lipid mass

is not directly measured with this method, unacceptable errors in lipid mass estimates may occur when testing relatively lean animals (Morton 1991, Voltura and Wunder 1998).

In contrast, we confirmed that using a multiple regression model to directly estimate lipid mass may be the best strategy when using EM-SCAN® (Voltura 1996). Using multiple regression, researchers should select the most parsimonious model without sacrificing accuracy in body composition estimates. In our study, the strong correlation between total body mass and fat-free mass ($r = 0.88$) and between dry (constant mass) body mass and lipid mass ($r = 0.86$) supported including total body mass as an essential regression parameter. In addition, the inclusion of a size parameter, to account for differences in body geometry (Fiorotto et al. 1987), was also necessary and included as in the equation of Voltura and Wunder (1998). In deriving our calibration equation, we were able to reduce the necessary parameters in the model to total body mass and the EM-SCAN® index. With this procedure, we could determine body composition using only body mass, body length without tail, and the EM reading. This multiple regression model accounted for 85.9 % of the variation in lipid mass with similar estimates of both lipid mass and percent body fat as the two-stage model. With the multiple regression model, the average error of lipid mass estimates was reduced to 1.55 ± 0.18 g. As a result, we believe this model to be highly effective in estimating lipid mass over a wide range of body compositions in meadow voles. This model is appropriate for estimates of lean wild-caught voles, which often contain between 3 to 9 % body fat, as well as for voles which maintain much higher body fat levels (as observed in the lab). Overall, concerns for the

high error in lipid mass estimates with lean field animals expressed by Voltura and Wunder (1998) are greatly reduced.

As expected, the meadow vole model derived for this study outperformed the other two models in estimating lipid mass and percent body fat in meadow voles (Figure 2.2; Table 2.4, 2.5). Regardless of which data set was used, the estimates of body composition using the meadow vole model were both reliable and consistent. The generic rodent model resulted in unacceptably high errors in estimates of lipid mass, ranging from 4.04 to 5.73 g (Figure 2.2). A 64 - 70 % improvement in average error of estimates resulted with the meadow vole model. Therefore, the generic rodent model would be most useful to estimate body composition in "fat" laboratory rats. In contrast, the prairie vole model had much smaller errors in its estimates than the generic rodent model. The average error in estimates of lipid mass varied by data set and ranged from 4.57 ± 0.42 g to 2.25 ± 0.54 g, with percent body fat errors ranging from 5 - 11 % (Figure 2.1; Table 2.4). With the meadow vole model, the smallest error in lipid mass estimates resulted with the "lean" subset, with the largest error associated with the "fat" subset. This was expected since the "fat" voles had a larger variance for all body composition parameters than the "lean" voles. In addition, the complete data set resulted in errors in lipid mass estimates intermediate to both subsets, reflecting an averaging of extremes in body composition of voles in the two data subsets. Comparing the average error in estimates of lipid mass between both vole models showed a 57 % improvement with the meadow vole model using the complete data set. Using the data subsets, the improvement with the meadow vole model over the prairie vole model ranged from 25 - 69 %.

The strength and flexibility of our model to accurately estimate body composition across a wide continuum was the result of including voles with a wide range of body compositions when deriving our calibration equation. As with all regression models, the relationship between the dependent and independent variables is strongest within the range of the data (Zar 1984). Extrapolations outside the range of data used to define the variable relationship may be less reliable. Voltura and Wunder (1998) recommended that EM-SCAN® SA-2 was unable to predict lipid mass of relatively lean animals with sufficient accuracy. This was true in their study of prairie voles, because the calibration equation was derived from wild-caught voles which were held in a lab for several months and had an average percent body fat of 20 %. Then, the use of this equation to estimate body composition on lean voles in the field (3 - 9 % body fat) resulted in high estimate errors. We believe that derivation of our meadow vole model assimilated these concerns and confirm that EM-SCAN® can estimate body composition to a level of accuracy acceptable for studies of comparatively lean small mammals.

We also believe that a more general "morph" regression calibration equation to estimate body composition with EM-SCAN® may also be worth consideration and reduce the need to derive species-specific equations under certain circumstances. It may be possible to derive a calibration equation for a specific species and apply this equation to other species with similar morphology and body composition dynamics. Such may be the case for voles, and in particular meadow voles and prairie voles. The general morphology of these species is nearly identical, with the only obvious differences in underside color and tail length. We confirmed our "morph" hypothesis by investigating the estimates of

lipid mass for both the meadow and prairie vole models using the "fat" data subset (Figure 2.1; Table 2.4). The "fat" subset had an average lipid mass of 10.41 ± 1.47 g and a percent body fat of 22.47 ± 2.60 % (Table 2.1). With this data subset, the average error in lipid mass estimates for the meadow vole model was 1.70 ± 0.25 g, while the prairie vole model had an average error of 2.25 ± 0.54 g for lipid mass. As a result, the difference in average error of 0.55 g between models results in a 25 % improvement realized by the meadow vole model. Although seemingly quite large, this improvement is rather insignificant when applied to relatively fat voles. Given a hypothetical "fat" vole with a total mass of 50 g, composed of 10 g body fat and 40 g of fat-free mass, the improvement of 0.55 g with the meadow vole model actually equates to a 5 % improvement in estimates of error. Therefore, we interpret this slight improvement in lipid mass estimates to negate the need to for a species-specific model in this case. Either model would be very appropriate if used to estimate body composition in voles which had a relatively high percent body fat, a condition which is prevalent in the lab. These results also suggest that "morph" models may be useful if applied when the expected estimates of body composition are similar to the range of body composition for animals used to derive the model. To highlight this precaution, we could apply the same two vole models to a hypothetical "lean" 50 g vole with 3 g of body fat and 47 g of fat-free mass, a common condition in the field. In this situation, the 0.55 g difference between models results in an 18 % improvement in the error in estimates of lipid mass. The increased improvement of the meadow vole model was due to the inclusion of 16 "lean" voles during model derivation. In contrast, the prairie vole model was attempting to estimate body composition of voles outside the range

of the data used in its derivation. Based on these results, we encourage researchers to consider general "morph" models if such models can meet research requirements. However, the extent of variation in morphology that may be included in a general "morph" model remains unknown and awaits further research.

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Table 2.1. Comparison of body composition parameters for the complete data set and two partial data subsets used in cross validation analyses of EM-SCAN®. Values are mean plus/minus one standard error (in parentheses).

	Complete Data n = 27	Partial Data "Lean" n = 16	Partial Data "Fat" n = 11
Total body mass (g)	47.07 (1.91)	47.91 (2.61)	45.85 (2.83)
Lipid mass (g)	5.92 (0.95)	2.84 (0.27)	10.41 (1.47)
Fat-free mass (g)	41.15 (1.97)	45.07 (2.53)	35.44 (2.32)
Percent lipid mass (%)	12.75 (1.91)	6.07 (2.53)	22.47 (2.60)
Percent fat-free mass (%)	87.25 (1.91)	93.97 (2.53)	77.57 (2.60)
Percent total body water (%)	63.28 (1.47)	68.37 (0.45)	55.89 (2.05)

Percent lipid mass (%) = (lipid mass/total body mass) x 100
Percent fat-free mass (%) = (fat-free mass/total body mass) x 100
Percent total body water (%) = ((wet total body mass - dry total body mass)/ wet total body mass) x 100

Table 2.2. Comparison of simple linear and multiple regression equations derived in this study to estimate body composition in meadow voles using EM-SCAN®. Values are mean plus/minus one standard error (in parentheses).

	Microtus pennsylvanicus Multiple regression	*Microtus pennsylvanicus* Simple Linear regression
Equation	LM = 3.733 + 0.957 (bm) - 0.684 (em2)	FFM = (em2 - 6.090)/1.381
R^2	85.9%	97.1 %
Prediction of lipid mass and percent body fat		
Complete data set; n = 27		
Average error, grams lipid	1.55 (0.18)	1.60 (0.18)
Average error, percent body fat	3.56 (0.50)	3.38 (0.50)
Partial Lean data set; n = 16		
Average error, grams lipid	1.44 (0.26)	1.52 (0.27)
Average error, percent body fat	3.24 (0.66)	3.42 (0.66)
Partial Fat data set; n = 11		
Average error, grams lipid	1.70 (0.25)	1.71 (0.23)
Average error, percent body fat	4.16 (0.86)	4.03 (0.78)

Average error, grams lipid = mean of absolute value (actual lipid mass (g) - estimated lipid mass (g))
Average error, percent body fat = mean of absolute value (actual % lipid - estimated % lipid)
Percent (%) lipid = (lipid mass/total body mass) x 100
bm = total body mass; em2 = EM index; LM = lipid mass; FFM = fat-free mass

Table 2.3. Comparison of body composition parameters for the complete data set, "fat" partial data subset, and prairie vole data set used in cross validation analyses of EM-SCAN®. Values are mean plus/minus one standard error (in parentheses).

	Complete Data n = 27	Partial Data "Fat" n = 11	Prairie Vole (Voltura & Wunder 1996) n = 15
Total body mass (g)	47.07 (1.91)	45.85 (2.83)	47.0 (1.7)
Lipid mass (g)	5.92 (0.95)	10.41 (1.47)	9.6 (0.8)
Fat-free mass (g)	41.15 (1.97)	35.44 (2.32)	37.4 (1.4)
Percent lipid mass (%)	12.75 (1.91)	22.47 (2.60)	20.4 (1.4)
Percent fat-free mass (%)	87.25 (1.91)	77.57 (2.60)	79.6 (1.4)
Percent total body water (%)	63.28 (1.47)	55.89 (2.05)	NR

Percent lipid mass (%) = (lipid mass/total body mass) x 100
Percent fat-free mass (%) = (fat-free mass/total body mass) x 100
Percent total body water (%) = (wet total body mass - dry total body mass) x 100
NR = not reported

Table 2.4. Comparison of average error in estimates of lipid mass and percent body fat for *M. pennsylvanicus* using equations derived for M. *pennsylvanicus* (this study) and *M. ochrogaster* (Voltura 1996). Values are mean plus/minus one standard error (in parentheses).

	Microtus pennsylvanicus Multiple regression (this study)	*Microtus ochrogaster* Multiple regression (Voltura & Wunder 1998)
Prediction of lipid mass		
Complete data set		
Average error, grams lipid	1.55 (0.18)	3.63 (0.40)
% improvement, grams lipid	57.3 %	
Average error, percent body fat	3.56 (0.50)	7.72 (0.77)
% improvement, percent body fat	53.9 %	
Partial Lean data set		
Average error, grams lipid	1.44 (0.26)	4.57 (0.42)
% improvement, grams lipid	68.5 %	
Average error, percent body fat	3.24 (0.66)	9.64 (0.77)
% improvement, percent lipid	66.4 %	
Partial Fat data set		
Average error, grams lipid	1.70 (0.25)	2.25 (0.54)
% improvement, grams lipid	24.4 %	
Average error, percent body fat	4.03 (0.78)	4.92 (1.08)
% improvement, percent body fat	18.1 %	

Average error, grams lipid = mean of absolute value (actual lipid mass (g) - estimated lipid mass (g))
Average error, percent body fat = mean of absolute value (actual % lipid - predicted % lipid)
% improvement = (estimate (*m.o.*) - estimate (*m.p.*))/ estimate (*m.o.*)

Table 2.5. Comparison of average error in estimates of lipid mass and percent body fat for *M. pennsylvanicus* using equations derived for M. *pennsylvanicus* (this study) and a generic rodent (EM-SCAN® SA-2). Values are mean plus/minus one standard error (in parentheses).

	Microtus pennsylvanicus Multiple regression	EM-SCAN® SA-2 Multiple regression
Prediction of lipid mass		
Complete data set		
Average error, grams lipid	1.55 (0.18)	4.73 (0.71)
% improvement, grams lipid	67.2 %	
Average error, percent body fat	3.56 (0.50)	11.83 (2.39)
% improvement, percent body fat	69.9 %	
Partial Lean data set		
Average error, grams lipid	1.44 (0.26)	4.04 (0.81)
% improvement, grams lipid	64.4 %	
Average error, percent body fat	3.24 (0.66)	9.76 (2.71)
% improvement, percent body fat	66.9 %	
Partial Fat data set		
Average error, grams lipid	1.70 (0.25)	5.73 (1.28)
% improvement, grams lipid	70.3 %	
Average error, percent body fat	4.03 (0.78)	14.84 (4.34)
% improvement, percent body fat	72.8 %	

Average error, grams lipid = mean of absolute value (actual lipid mass (g) - estimated lipid mass (g))
Average error, percent body fat = mean of absolute value (actual % lipid - predicted % lipid)
% improvement = (estimate (EM-SCAN) - estimate (*m.p.*))/ estimate (EM-SCAN)

Figure 2.1. Relationship between fat free mass and EM index.

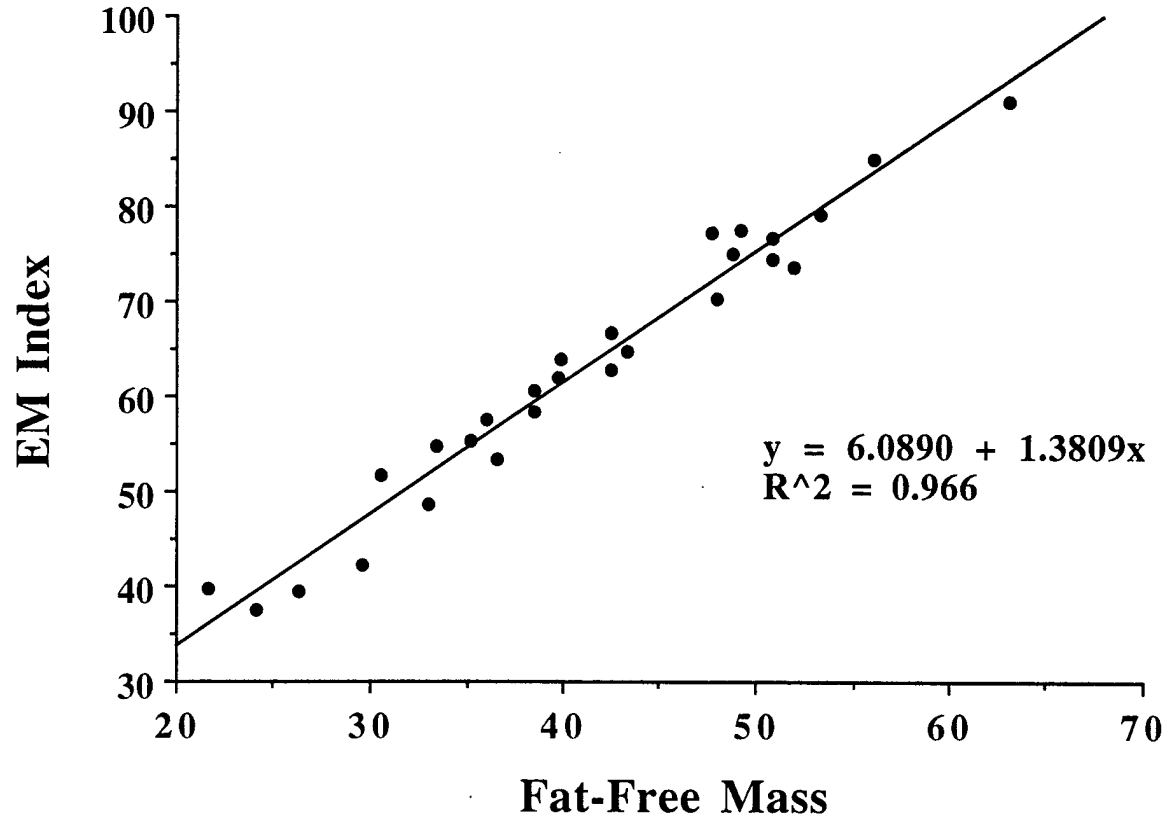
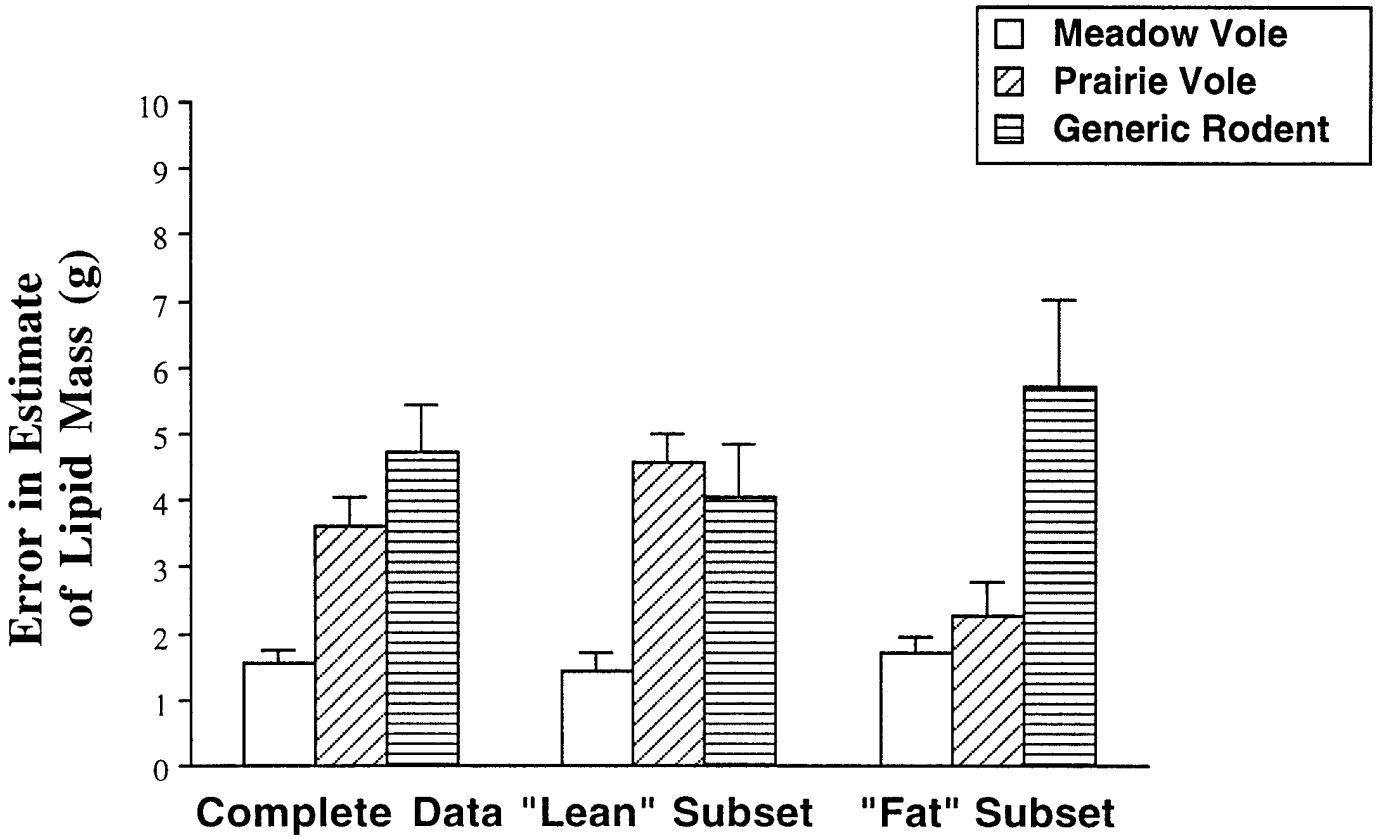


Figure 2.2. Error in the estimates of lipid mass between calibration models. Values are mean plus/minus one standard error (error bars).



Chapter 3

Use of the EM-SCAN® device for estimating body composition in the meadow vole, *Microtus pennsylvanicus*: effects of food, water and anesthesia

INTRODUCTION

In many studies of small mammals, body mass is used as an estimate of body size (Iskjaer et al. 1989), seasonal variation in size (Barbehenn 1955; Iverson and Turner 1974), or growth (Barbehenn 1955; Brown 1973; Chitty 1952; Iverson and Turner 1974). However, body mass has been shown to be variable, especially when animals are retained in livetraps. A single livetrap capture can decrease total body mass up to 10 % as shown in meadow voles (*Microtus pennsylvanicus*) (Barbehenn 1955) and in deer mice (*Peromyscus maniculatus*) (Kaufman and Kaufman 1989, 1994). Additional studies of small mammals have suggested that individuals will continue to lose mass with repeated livetrap captures over short time periods (Brown 1973; Iverson and Turner 1974; Korn 1987; Slade 1991; Slade and Iskjaer 1990). Body mass losses from 4 - 9 % were found in prairie voles (*M. ochrogaster*) captured repeatedly over a three day period (Slade 1991; Slade and Iskjaer 1990). These results suggest that when using body mass as a categorical variable, the mass fluctuations create additional uncertainty and inflate the variation in one's data. The potential causes for loss of body mass could be due to capture stress and handling, food deprivation and/or dehydration due to trap confinement, lipid or fat-free tissue catabolism or any combination thereof.

To date, no one has reported the effects of livetrap retention on the body composition of a captured animal. Thus, we used a repeatable, non-invasive technique (EM-SCAN®) to estimate changes in body composition of meadow voles due to different conditions while retained within a trap. In many studies, livetrapping involves setting traps at dusk and then checking them at dawn for captures. Therefore, depending upon the season and actual time of capture, a vole could hypothetically spend up to 14 hours in a trap during winter and 8 hours in the summer under such protocols. During trap retention, the captured animal often has only the food in the trap and water within that food for subsistence. As a result, the presence or absence of food and water can influence energy availability, gutfill, and hydration in the captured animals. Added physiological stress may also be associated with ambient temperature. In summer, high ambient temperatures may result in increased dehydration. In contrast, higher thermogenic demands associated with lower ambient temperatures may be found in winter. Thus, we designed our experiment to investigate the potential effects of gutfill and hydration, along with ambient temperature on body composition.

The EM-SCAN ® device has been used as a rapid, noninvasive tool to estimate body composition in live small mammals and birds (Bachman 1994; Castro et al.. 1990; Morton et al. 1991; Osborne et al. 1996; Scott et al. 1991, 1996; Skagen and Knopf 1993; Voltura 1996; Voltura and Wunder 1998; Walsberg 1988). With EM-SCAN®, the accuracy and reliability of body composition estimates are dependent upon both a consistent measurement protocol, as well as a constant physiological state in the subject. Physiological conditions such as body temperature, hydration state, and gutfill have been

identified as potential causes for estimation error using EM-SCAN® technology (Bachman 1994; Voltura 1996; Voltura and Wunder 1998; Walsberg 1988). Walsberg (1988) suggested that a decrease in hydration will result in a lower estimate of fat-free mass with EM-SCAN®. Since dehydration will influence divalent ion form (ionic vs. molecular) in the body, EM-SCAN® will estimate less fat-free mass. Because fat-free mass plus lipid mass equals total body mass, a lower estimate of fat-free mass has an inverse effect on lipid mass. EM-SCAN® estimates of fat-free mass are also influenced by the amount of food within the gastrointestinal tract of an animal (Bachman 1994; Voltura 1996; Voltura and Wunder 1998). Voltura and Wunder (1998) showed that ingesta (lab chow) is read by the EM-SCAN® device as fat-free mass. Bachman (1994) cautioned that high water and mineral (potassium ion) levels in gutfill ingesta can complicate the EM-SCAN® reading. She confirmed the need to account for gutfill, and in particular, food types and the sporadic, relatively large meals consumed by carnivores.

Endotherm body temperature is generally stable enough not to be a source of intermeasurement variation with EM-SCAN®. However, body temperature may vary due to stress, activity, or experimental technique and handling. Walsberg (1988) alleged that changes in body temperature greater than 4° C could produce up to 5 % error in body composition estimates by EM-SCAN®. In addition, significant variation in the estimates of fat-free mass was found in laboratory rats 80 minutes after anesthesia application (Tobin & Finegood 1995). Tobin and Finegood (1995) suggested that the change in estimates of fat-free mass was most likely due to changes in core body temperature.

In our study, we kept voles in Sherman livetraps for eight hours and manipulated the presence or absence of food and water to investigate whether gutfill and dehydration would influence body composition. In addition, we varied ambient temperature (5° C vs. 23° C), to determine if environmental conditions would elicit a different body composition response. Lastly, we conducted an experiment to evaluate the effects of anesthesia on body temperature, and thus EM-SCAN® estimates of body composition.

MATERIALS AND METHODS

Effects of food and water during trap retention at 23 °C

Twenty-four meadow voles approximately two months of age were selected from a breeding colony established at Colorado State University. Within the breeding colony, voles were housed individually (28 x 18 x 13 cm) with lab chow (Lab Diet 5001, PMI Feeds) and water *ad lib.* and kept at 23° C with a photoperiod of 16L:8D. Voles were initially ranked by mass and were randomly assigned to one of three experimental conditions (control, group A, group B) in groups of three. This procedure was repeated for each additional three animal group and continued until all voles were assigned, resulting in a block design. Body composition of all voles was measured at 0800 on day 1 using EM-SCAN® procedures described by Voltura and Wunder (1998). Animals were then returned to their cages to recover from anesthesia and were provided lab chow and water *ad lib.* At 2300 on day 1, voles were placed into their respective treatments. All were at 23° C. Control voles remained within their individual cages with lab chow and

water *ad lib.* Group A voles were placed individually within a Sherman livetrap (22.9 x 7.6 x 8.9 cm) for eight hours with no food or water. Group B voles were also held in Sherman livetraps for eight hours, but given a premeasured amount of lab chow and no water. Photoperiod lights out occurred at 2300 with lights on at 0700 the following day, consistent with conditions at the breeding colony. On day 2 at 0700, body composition was remeasured for all voles to investigate changes in body composition over the trap night. Since we expected the greatest change in body composition to result in Group A and our Animal Care protocol required that we get them back onto food and water as soon as possible, we measured group A first, followed by group B, and lastly, control. All voles were measured within two hours.

On day 3, all voles were given *ad lib.* food and water to recover from the initial experiment. At 0800 on day 4, body composition was again measured to see whether it had returned to that present at day 1. If recovery was not complete, we extended the recovery another day. However, all voles returned to prior body composition levels within one day. Voles were then returned to their cages with lab chow and water *ad lib.* At 2300 on day 4, groups A and B were assigned to different manipulations. All were at 23° C. Control voles remained as controls and were held in their individual cages with *ad lib.* food and water. Voles in group A were placed individually in a Sherman livetrap for eight hours and given water (50 ml in a ceramic container, 6 cm opening) and no food. Group B voles were maintained under identical conditions as group A, but given a premeasured amount of apple instead of water. A final measure of body composition was completed the following day at 0700, using the same order of measure previously

described. Food, apple, or water consumption was calculated as the difference between initial mass and mass after the eight hour period. We accounted for mass change due to evaporation or condensation by placing two premeasured samples each of food, apple, and water inside an unoccupied Sherman livetrap within the environmental chamber. This change in mass was then subtracted from each specific intake rate.

Body composition data collected included measurements of total body mass (Ohaus E400D) and EM-SCAN® estimates. Percent lipid and percent fat-free mass were calculated by dividing lipid mass and fat-free mass estimates by total body mass. In addition, changes in total body mass, lipid mass, and fat-free mass were calculated by subtracting the appropriate mass value after the eight hour experimental period from its original value.

Effects of food and water during trap retention at 5 ° C

Procedures were identical to those described above, except that ambient temperature was held at 5° C rather than 23° C. In addition, we used a different group of 24 voles from the CSU breeding colony. To maximize thermal stress potential, the voles were not acclimated at 5° C before experimentation. Livetraps were placed into the environmental chamber (5° C) at 0800 until the voles were placed within the traps at 2300 to ensure cold conditions (near 5° C) at the onset of vole occupancy .

Effect of supplemental apple after trap occupancy

In the first two experiments, we investigated the differential effects of food and water availability on body composition while an animal was in a trap. In this experiment, we attempted to simulate field trap conditions where food or water may not be available or an animal does not eat under such circumstances. Here, we gave no food or water for eight hours and then provided apple as a substitute for both food and water in an attempt to bring gutfill to "normal" levels after animals have been in a trap. In this study, we used a different group of 24 voles from the CSU breeding colony and assigned them to a control (food and water *ad lib.*) and two experimental groups as previously described. All groups were held at 5° C. Group A voles were held individually within a Sherman livetrap for eight hours and received no food or water. Group B conditions were identical to group A except that a premeasured amount of apple was given to the voles after trap retention for eight hours. Group A and control were measured for body composition after eight hours. Group B was provided apple for an additional three hours to rehydrate and provide gut-fill (if consumed) and then were measured with EM-SCAN®.

Apple intake was measured as the difference between initial apple mass and mass after the three hour period of "apple availability". Change in apple mass due to evaporation after three hours was subtracted from intake values as previously described.

Immediately after completing the final EM-SCAN® measures, the 24 voles used in this study were euthanized with methoxyfluorane (Metophane, Mundelein, IL) overdose. To investigate the extent of dehydration, total body water was determined. Each vole was shaved to remove fur and the gastrointestinal tract (stomach to anus) was removed by

dissection. The carcass, fur, and gastrointestinal tract were then dried to a constant mass at 60° C using a forced air convection drier. The mass difference between initial total body mass (wet) and constant total body mass (dry) were used to calculate total body water. In addition, chemical (ether) extraction of body fat was completed by a contracted lab at the University of Western Ontario (UWO), London, Ontario, Canada using a modified sohxlet procedure. The dried carcass and gastrointestinal tract with contents were ground in a coffee grinder to ensure homogeneity and the entire contents for each vole were used in the sohxlet procedure. The lipid mass value measured by UWO was then compared to the estimated lipid mass value from EM-SCAN® using regression techniques to confirm estimation accuracy.

Effects of anesthesia-induced change in body temperature on estimates of body composition by EM-SCAN®

Fourteen meadow voles were captured in June 1996 at the United States Air Force Academy, El Paso County, Colorado. After capture, voles were individually housed in small cages (28 x 18 x 13 cm) with lab chow (Lab Diet 5001, PMI Feeds) and kept at 23° C and 16L:8D. Body composition of voles was measured using procedures previously described by Voltura and Wunder (1998). Once anesthetized and motionless, rectal body temperature was measured by inserting a thermocouple to 1 cm depth. The thermocouple was removed and the voles were then placed within the EM-SCAN® device. Additional body temperature and body composition measures were repeated every five minutes for fifteen minutes. We chose a 15 minute time period because the maximum time a vole would ever be anesthetized prior to completing EM-SCAN® measurements

under our procedures would be 15 minutes. In addition, our prior experience showed that voles begin to awaken from the initial anesthesia application after about 15 minutes. In this experiment, no additional anesthesia was administered during the fifteen minute period. If a subject failed to remain motionless or aroused from anesthesia during the EM-SCAN® measurement procedure, data for that individual were removed from the analysis. Of the fourteen voles which began the experiment, eleven completed three estimates over a 10 minute period, with eight of these eleven able to complete an additional estimate at 15 minutes. We performed separate analyses of both the 10 minute and 15 minute periods.

Statistics

For all comparisons between groups, statistical significance was set at $p < 0.05$. In the livetrap experiments, comparisons of body composition parameters between groups were performed using ANOVA (SAS, proc glm) (SAS Institute, Inc., 1989) and tested with least squared differences. In the apple supplementation livetrap experiment, ANOVA comparisons between groups for total body water and the difference between estimated lipid mass and measured lipid mass were also completed. A regression with estimated lipid mass as the independent variable and measured lipid mass as the dependent variable was also done to confirm EM-SCAN® estimate accuracy.

In the temperature effects experiment, a repeated measures ANOVA (SAS, proc mixed) (SAS Institute, Inc., 1989) with time (5 minute periods) as the fixed effect and subject as a random effect was performed for three body composition parameters: total

body mass, lipid mass, fat-free mass, and for body temperature. In addition, change in each parameter, as well as body temperature, were calculated to highlight the magnitude of change over time from original conditions. These included the change from start to time 1 (5 minutes), start to time 2 (10 minutes), and start to time 3 (15 minutes). Change values were calculated by subtracting a parameter value at the start from the resulting value at time 1, time 2, and time 3 respectively.

RESULTS

Body composition change at 23 °C

Significant differences between groups were present for total body mass ($F_{4,35}$ = 8.25; p = 0.0001), fat-free mass ($F_{4,35}$ = 7.98; p = 0.0001), and percent fat-free mass ($F_{4,35}$ = 7.96; p = 0.0001). However, significant differences between groups for lipid mass or percent lipid mass were not evident. Voles with no food or water, as well as no water but food showed the greatest loss in body mass and fat-free mass. Smaller losses in body and fat-free mass resulted for voles in the water and no food condition. When apple was available, no significant difference from control for any parameter was evident (Figure 3.1, Table 3.1). When provided no food or water, voles lost 3.62 ± 0.56 g of body mass, due entirely to losses of fat-free mass (3.71 ± 0.69 g). When given food but no water, similar results occurred with losses of 3.58 ± 0.65 g of body mass and 3.22 ± 0.53 g of fat-free mass. With no food but water, voles lost 3.22 ± 0.39 g of body mass with lesser losses in fat-free mass (1.59 ± 0.45 g). When given apple, the change in body composition did not

differ significantly from control, with minimal losses in body mass (1.06 ± 0.46 g) and fat-free mass (0.56 ± 0.37 g). Overall, losses in total body mass were due mainly to losses in fat-free mass. When water was available voles had smaller losses in body and fat-free mass. There was no significant difference in lipid mass between groups. Thus, when given food without water in a livetrap for eight hours, voles still lose as much body mass as when given nothing; whereas apple allows animals to maintain body mass.

In the food and no water condition, lab chow consumption was 0.54 ± 0.05 g with a range from 0.42 to 0.82 g. Water intake in the water but no food condition was 3.75 ± 1.01 ml with a range from 0.50 to 8.50 ml. Apple intake (wet) was 9.26 ± 1.46 g with a range from 4.94 to 16.97 g. Given these results, it appears that with no water, food (as lab chow blocks) is of little value and very little is eaten.

Body composition change at 5 °C

There were significant differences between groups for total body mass ($F_{4,35}$ = 16.42; p = 0.0001), fat-free mass ($F_{4,35}$ = 7.00; p = 0.0003), and percent fat-free mass ($F_{4,35}$ = 6.83; p = 0.0004) (Figure 3.2). In contrast to changes observed at 23° C, greater losses in lipid mass occurred, often equaling or exceeding losses in fat-free mass. Losses of lipid mass in the experimental groups ranged from 1.47 ± 0.37 g to 2.31 ± 0.23 g, values 5 times greater than those observed at 23° C. Even though losses in lipid mass were greater at 5° C, changes in lipid mass or percent body fat between groups was similar suggesting a consistent effect due to temperature, regardless of treatment. Overall, losses

in body mass and fat-free mass were also greater at 5° C than those observed in similar groups exposed to 23° C. With no food or water, voles lost 4.51 ± 0.54 g of body mass and 2.80 ± 0.65 g of lean mass. When given food but no water, similar mass losses occurred: 4.41 ± 0.41 g of body mass and 2.13 ± 0.80 g of fat-free mass. Voles with water but no food lost less body mass (3.76 ± 0.49 g) and 2.30 ± 0.72 g of fat-free mass. With apple, changes in body composition were not statistically different from control, with losses of 1.23 ± 0.37 g of body mass and 0.95 ± 0.33 g of lean mass (Figure 3.2, Table 3.2). Consistent with changes in body composition at 23° C, both food or water deprivation, as well as providing food only resulted in the largest changes in body mass; whereas water availability produced less body mass loss. When given apple, voles maintained body mass in a trap at 5° C for eight hours.

With food but no water, intake of lab chow was 0.68 ± 0.17 g with a range from 0.10 to 1.39 g, similar to intake at 23° C. If given water but no food, water intake was greater than at 23° C (6.50 ± 1.36 ml with a range from 1.50 to 14.50 ml). Apple consumption (wet) was also higher than at 23° C and averaged 13.46 ± 1.12 g with a range from 9.37 to 18.28 g.

Body composition change when given apple after deprived of food and water for eight hours

Total body mass ($F_{2,21}$ = 15.28; p = 0.0001), fat-free mass ($F_{2,21}$ = 3.86; p = 0.0373), percent fat-free mass ($F_{2,21}$ = 4.46; p = 0.0244) all showed significant differences between groups. Significant differences between groups for lipid mass and percent lipid

mass were not observed but all groups lost lipid mass and decreased percent body fat. As expected with low ambient temperatures (5° C), all voles lost body fat and both experimental groups lost similar amounts of fat-free mass (Figure 3.3, Table 3.3). When deprived of food or water for eight hours, voles lost 3.10 ± 0.56 g of body mass and 1.15 ± 0.87 g of fat-free mass. Voles deprived of food and water for eight hours and then given apple for three hours still showed losses in body mass (3.04 ± 0.32 g), but lost smaller amounts of fat-free mass (0.77 ± 0.71 g). As a group, the availability of apple did not affect the change in body composition compared to the group with no food or water. If a vole consumed little or no apple over the three hour period, this vole was actually without food or water for eleven hours. In contrast, when voles ate apple, they received the benefits of rehydration and increased gut-fill, and therefore, lessened the loss of fat-free mass. Therefore, the change in body composition for the group given apple was actually an average of two extreme subset effects within the group.

Apple intake ranged from 0.00 to 3.37 g with an average of 1.32 ± 0.56 g. Three voles did not consume any apple, with two of these voles showing decreased fat-free mass from original conditions. In addition, three voles consumed at least 2.65 g or more of apple, with two of these voles having increased fat-free mass over the same time period. The remaining two voles consumed intermediate amounts of apple (0.13 g and 0.94 g respectively) and both had lost fat-free mass. Thus, 66 % of voles who ate adequate amounts of apple either maintained or increased fat-free mass. However, when eating none or small amounts (< 1.0 g) of apple, fat-free mass decreased in 4 of 6 voles. Thus, we conclude that the use of apple to rehydrate and provide gut-fill after trap retention

reduces overall body composition change provided that animals eat the apple, but not all do.

The voles used in this experiment were laboratory reared and had high body fat (20 to 25 %) compared to voles in the wild (3 to 8 %). Since fat contains less water than lean tissue (Walsberg 1988), total body water is much less in "fat" lab reared voles than those in the wild. In our experiment, control voles (assumed to be fully hydrated) had a total body water of 52.75 ± 1.49 % with a range of 48 to 60 %. In contrast, when deprived food and water, hydration levels were reduced to 48.00 ± 1.71 % with a range from 42 to 56 %. This 5 % reduction in total body water equates to 2.5 g of water loss in a 50 g vole.

Accuracy of EM-SCAN® estimates of lipid mass

Comparing the estimates of lipid mass from EM-SCAN® for voles in the apple supplementation experiment to lipid mass levels measured by chemical extraction, no significant differences were found within a group ($p > 0.05$) or between groups ($F_{2,21} = 0.24$; $p = 0.7916$) (Figure 3.4). For percent lipid mass, there was also no significant difference within or between groups ($F_{2,21} = 0.10$; $p = 0.9025$). In a regression with the EM-SCAN® estimate for lipid mass as independent variable and lipid mass measured by chemical extraction as the dependent variable, the resulting R^2 value was .952 (Figure 3.5).

Effects of anesthesia induced body temperature change on EM-SCAN® device estimates

Analysis of the eight voles measured over the 15 minute period while anesthetized showed no significant difference in estimates of lipid mass ($F_{3,21} = 0.89$; $p = 0.4647$), fat-free mass ($F_{3,21} = 0.89$; $p = 0.4647$), percent lipid mass ($F_{3,21} = 1.16$; $p = 0.3480$), and percent fat-free mass ($F_{3,21} = 1.16$; $p = 0.3480$) (Figure 3.5). In contrast, significant variation over time was present for body temperature ($F_{3,21} = 132.62$; $p = 0.0001$), which decreased $3.08 \pm 0.26°$ C in 10 minutes and $3.96 \pm 0.35°$ C after 15 minutes. Consistent estimates of body composition parameters were evident throughout all time periods (Figure 3.6).

For the 11 voles measured over a 10 minute period, estimates of lipid mass ($F_{2,20} = 1.46$; $p = 0.2557$), fat-free mass ($F_{2,20} = 1.46$; $p = 0.2557$), percent lipid mass ($F_{2,20} = 1.54$; $p = 0.2390$), and percent fat-free mass ($F_{2,20} = 1.54$; $p = 0.2390$) also showed no significant variation (Figure 3.7). A significant difference over time was present for body temperature ($F_{2,20} = 120.83$; $p = 0.0001$), which decreased $3.00 \pm 0.22°$ C in the 10 minute period.

DISCUSSION

Effects of trap retention on body composition

When retained in a Sherman livetrap for eight hours under conditions which restrict food, water, or both, meadow voles lose significant amounts of body mass

regardless of ambient temperature. Losses of body mass up to 7 % occurred at 23° C and losses increased to 8.5 % when exposed to 5° C. These results are consistent with mass losses observed when small mammals are captured once (Barbehenn 1955; Kaufman and Kaufman 1989, 1994) or repeatedly (Brown 1973; Iverson and Turner 1974; Korn 1987; Slade and Iskjaer 1990) over 1-3 days. However, in none of these field studies has anyone measured the change in body composition associated with the loss in body mass. In our study at 23° C, the change in total body mass was due primarily to losses of fat-free mass, especially when restricted from food and/or water. However, when voles received apple or *ad lib.* food and water, losses of fat-free mass were smaller, suggesting that fat-free mass loss is due to gutfill. At 5° C, voles lost similar amounts of fat-free mass compared to 23° C.

In contrast, they lost an additional 2 grams of lipid mass, suggesting they are burning body fat to maintain body temperature. A ranking based on conditions which result in the greatest loss in body mass is: 1) no food or water; 2) food and no water; 3) water and no food; 4) apple; and 5) food and water (control). This ranking pattern is consistent, regardless of ambient temperature stress. When voles were not given food, or did not eat food, larger decreases in body mass and fat-free mass occurred. This may be due to the lack of gutfill. Voltura and Wunder (1998) reported that gutfill is estimated by EM-SCAN® as fat-free mass. Therefore, the lack of ingesta after eight hours without food would result in lower estimates of fat-free mass. It is also possible that dehydration also contributed to the losses in body mass and fat-free mass, although to a lesser extent. However, we did not measure that.

When thermal stress was minimal (23° C), changes in lipid mass were very small (Figure 3.1). However, by lowering ambient temperature to 5° C, greater losses (2 to 3 times) in total body mass and lipid mass resulted (Figure 3.2, 3.3). Even voles given *ad lib.* food and water lost body fat at 5° C, yet they gained fat-free mass. This suggests that these voles ate more (estimated as increased fat-free mass) but couldn't eat fast enough to meet energy demands and so they catabolized body fat similar to restricted experimental groups. This reduction in body fat suggests that body fat is being catabolized to meet greater thermogenic demands. At both 23° C and 5° C, when food was available and water was not, food intake was minimal. In addition, voles increased intake under colder conditions. At 23° C, food consumption by voles in livetraps was 0.54 ± 0.05 g , with voles eating significantly more food at 5° C (0.68 ± 0.17 g). Voles with *ad lib.* food and water ate 2.04 ± 0.43 g at 23° C and 3.22 ± 0.17 g at 5° C over the same period, consistent with previous studies within our lab where voles maintained on *ad lib.* food and water at 23° C consumed around 6 to 8 g of food per 24 hour period. However, intake rates for voles deprived of water were much lower over the eight hour period. This suggests that the lack of available water probably precluded eating dry food. As a result, when water was not available, voles did not consume food and would have a lower gutfill level (which results in lower fat-free mass estimates), as shown by Voltura and Wunder (1998).

When water was available (as part of an apple or by itself), losses in body mass and fat-free mass were markedly less than when water was unavailable. At 23° C, water intake was 3.75 ± 1.01 ml and 6.50 ± 1.36 ml at 5° C. This suggests that improved

hydration will decrease losses in body mass. Increased hydration may also influence ion concentration and result in higher estimates of fat-free mass by EM-SCAN®. The effects of water on body composition change are further magnified when apple was available for the entire eight hour period. There was no significant difference in body composition between the voles fed apple for eight hours and those in the control, regardless of ambient temperature (Figure 3.1, 3.2). When voles ate apple, losses in total body mass and fat-free mass were significantly less than when water was provided but not food. We also interpret this difference as the effects of gutfill, since both groups received water (in some form) to rehydrate. Thus, both food and water are essential to maintain body composition. Overall, apple can decrease changes in body mass and fat-free mass by increasing gutfill and rehydrating captured animals. These results support the use of apple as a potential trap bait if investigators are interested in body mass of captured animals. Losses in body mass and changes in body composition due to food and water deprivation during trap retention can be reduced by using apple in trapping protocols.

When apple was given to voles after an eight hour trap retention, voles which ate greater than 2.65 g of apple lost less body mass and fat-free mass than voles which failed to eat. Reasons why voles failed to eat apple when available remains unknown. However, we believe that if using dry baits such as oats, oats with peanut butter, or lab chow during livetrapping, one should expect significant decreases in body mass and fat-free mass in the captured animal if retained in the trap for at least eight hours. However, if one provides apple immediately upon capture or if apple is used as a trap bait, changes in body composition can be reduced by restoring hydration relatively quickly (if the apple is eaten).

Temperature effects

When animals are placed under anesthesia, core body temperature decreases over time, but did not exceed losses greater than 4° C. Our results suggest that concerns for the effects of decreasing body temperature on the estimates of body composition with EM-SCAN® are unfounded, if measurements are completed within 15 minutes. Walsberg (1988) cautioned that changes in body temperature of more than 4° C will change estimates of body composition. In our study, up to 15 minutes after anesthesia application, body temperature did not drop more than 4° C. Thus, all other factors being equal, one can be reasonably certain that EM-SCAN® will be unaffected by changes in body temperature when estimates of body composition are completed before body temperature changes exceed 4° C. Our results further confirm that the protocol used in our study can achieve reliable and unbiased estimates of body composition because all measures are completed within 15 minutes.

Whether other species which vary significantly in body mass or morphology will respond similarly to anesthesia is unknown. Tobin and Finegood (1995) found errors of 3.5 % in estimates of fat-free mass at 4 minutes post anesthesia. After 80 minutes, significant differences in estimates of both fat-free mass (198 g before to 180 g after) and lipid mass (12.9 before to 21.1 g after) were evident and alleged to be due to changes in body temperature. One can determine if changes in body temperature due to anesthesia are warranted for a particular species by measuring changes in core body temperature after applying anesthesia for a specified period of time. If body temperature reductions exceed

4° C over the period of the EM-SCAN® measurement protocol, then estimates of fat-free mass may be biased.

Accuracy of EM-SCAN® estimates of lipid mass

Our study confirms that the EM-SCAN® device estimates lipid mass accurately under conditions of varying gutfill and hydration. Although estimates of lipid mass by EM-SCAN® were around one gram less than measured lipid levels in all groups, we found no significant differences between lipid mass levels within each group (Figure 3.4). In addition, the strong relationship ($R^2 = 0.952$) between the estimates of lipid mass and measured levels further confirm EM-SCAN® accuracy. The changes in hydration and gutfill in our manipulations produced changes in the estimates of fat-free mass by the EM-SCAN® device consistent with performance previously shown (Voltura and Wunder 1998; Walsberg 1988). These results provide further support for the use of EM-SCAN® in body composition studies and its ability to provide reliable and accurate data.

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Table 3.1. Effects of trap retention on body composition at 23° C. Values reported are mean plus/minus one standard error (in parentheses). Different letters indicate significant differences between groups ($p < .05$). Negative values indicate a loss in parameter from its original condition before trap retention.

Condition	% Mass Change	% Lipid Mass Change	% Fat-free Mass Change
Control	-1.17^{A} (0.89)	-0.26^{A} (1.59)	-1.44^{A} (1.23)
Exp #1A No Food, No water	-6.58^{B} (0.72)	4.25^{A} (6.41)	-9.00^{B} (1.42)
Exp #1B Food, No Water	-6.87^{B} (1.13)	2.53^{A} (9.16)	-8.46^{B} (1.43)
Exp #1C Water, No Food	-6.15^{B} (0.62)	-6.51^{A} (6.25)	-4.40^{A} (1.31)
Exp #1D Apple	-1.80^{A} (0.84)	-7.47^{A} (4.26)	-1.53^{A} (1.02)

** change = ((percent $value_{original}$ - percent $value_{after\ trap}$) / percent $value_{original}$) * 100

Table 3.2. Effects of trap retention on body composition at 5° C. Values reported are mean plus/minus one standard error (in parentheses). Different letters indicate significant differences between groups ($p < .05$). Negative values indicate a loss in parameter from its original condition before trap retention.

Condition	% Mass Change	% Lipid Mass Change	% Fat-Free Mass Change
Control	-2.16^{A} (0.47)	-7.44^{A} (3.21)	-0.77^{AC} (0.80)
Exp #1A No Food, No water	-8.44^{B} (0.84)	-11.65^{AB} (5.06)	-6.80^{B} (1.11)
Exp #1B Food, No Water	-8.64^{B} (0.61)	-26.07^{B} (10.08)	-4.62^{AB} (2.15)
Exp #1C Water, No Food	-7.64^{B} (1.16)	-10.74^{AB} (2.25)	-6.24^{B} (2.01)
Exp #1D Apple	-2.66^{A} (0.88)	-16.68^{AB} (2.91)	2.36^{C} (0.79)

** change = ((percent $value_{original}$ - percent $value_{after\ trap}$) / percent $value_{original}$) * 100

Table 3.3. Effects of trap retention and supplemental apple on body composition at 5° C. Values reported are mean plus/minus one standard error (in parentheses). Different letters indicate significant differences between groups ($p < .05$). Negative values indicate a loss in parameter from its original condition before trap retention.

Condition	% Mass Change	% Lipid Mass Change	% Fat-free Mass Change
Control	-0.79[A] (0.52)	-15.39[A] (3.05)	4.16[A] (1.44)
Exp #3A No Food, No water	-6.28[B] (0.81)	-12.46[A] (2.92)	-3.31[B] (2.24)
Exp #3B No Food, No Water, Apple	-6.61[B] (0.88)	-18.38[A] (2.83)	-2.45[B] (2.04)

** change = ((percent $value_{original}$ - percent $value_{after\ trap}$) / percent $value_{original}$) * 100

Figure 3.1. Effects of trap conditions on body composition of voles at 23° C. Values are mean plus/minus one standard error (error bars). Different letters indicate significant difference between groups ($p < .05$). Negative values indicate a decrease in the specific parameter after trap retention.

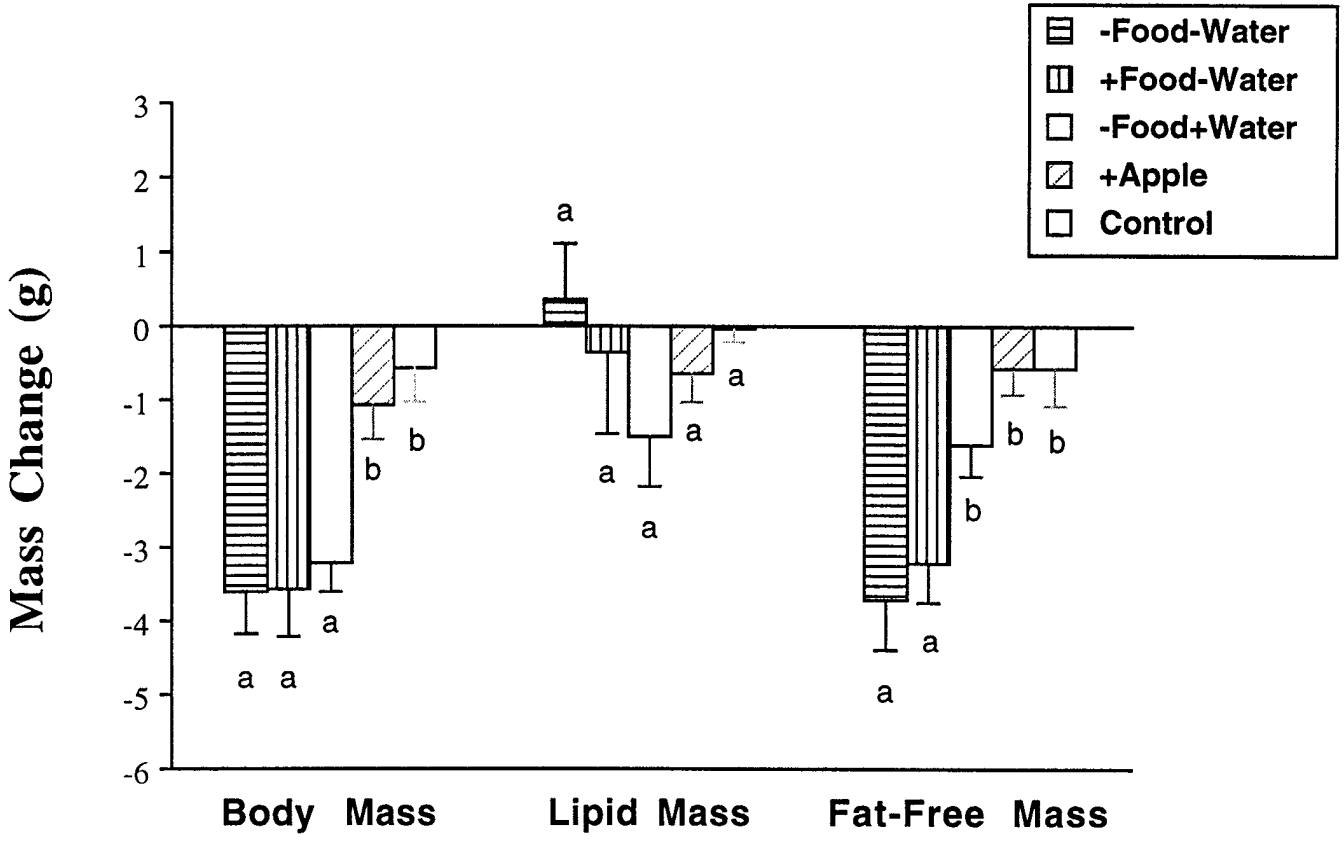


Figure 3.2. Effects of trap conditions on body composition of voles at 5° C. Values are mean plus/minus one standard error (error bars). Different letters indicate significant differences between groups ($p < .05$). Negative values indicate a decrease in the specific parameter after trap retention.

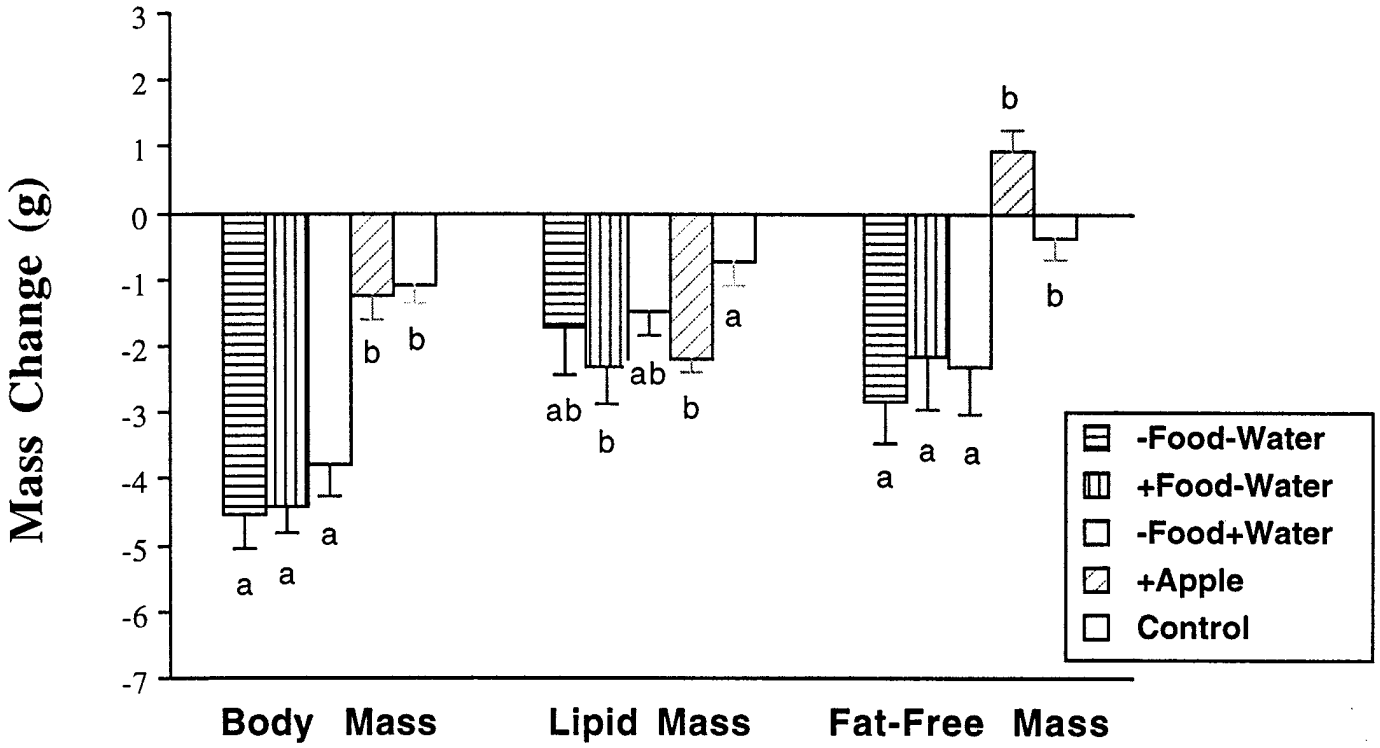


Figure 3.3. Effects of trap conditions and supplemental apple on body composition of voles at 5° C. Values are mean plus/minus one standard error (error bars). Difference letters indicate significant differences between groups ($p < .05$). Negative values indicate a decrease in a specific parameter after trap retention.

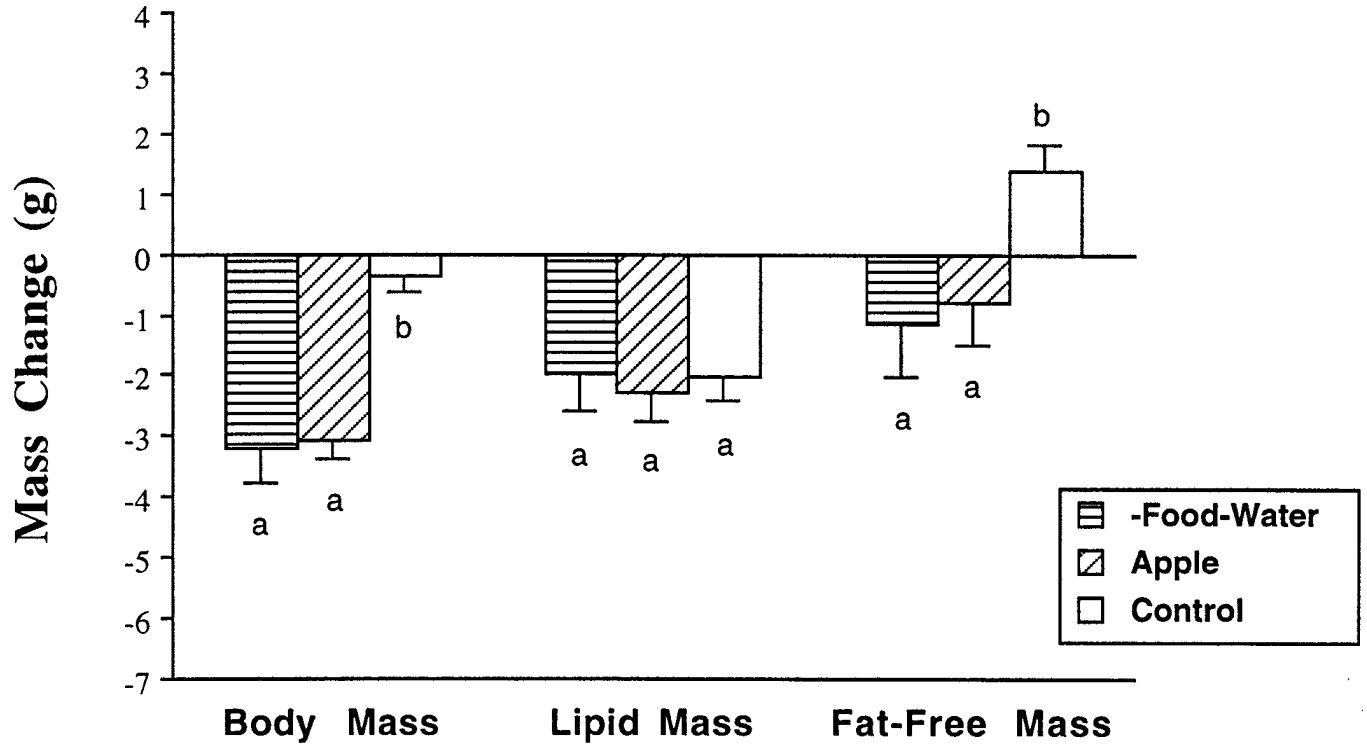


Figure 3.4. Comparison of lipid mass levels estimated by EM-SCAN and lipid mass levels measured by chemical extraction. Values are mean plus/minus one standard error (error bars). Different letters indicate significant differences within each group ($p < .05$).

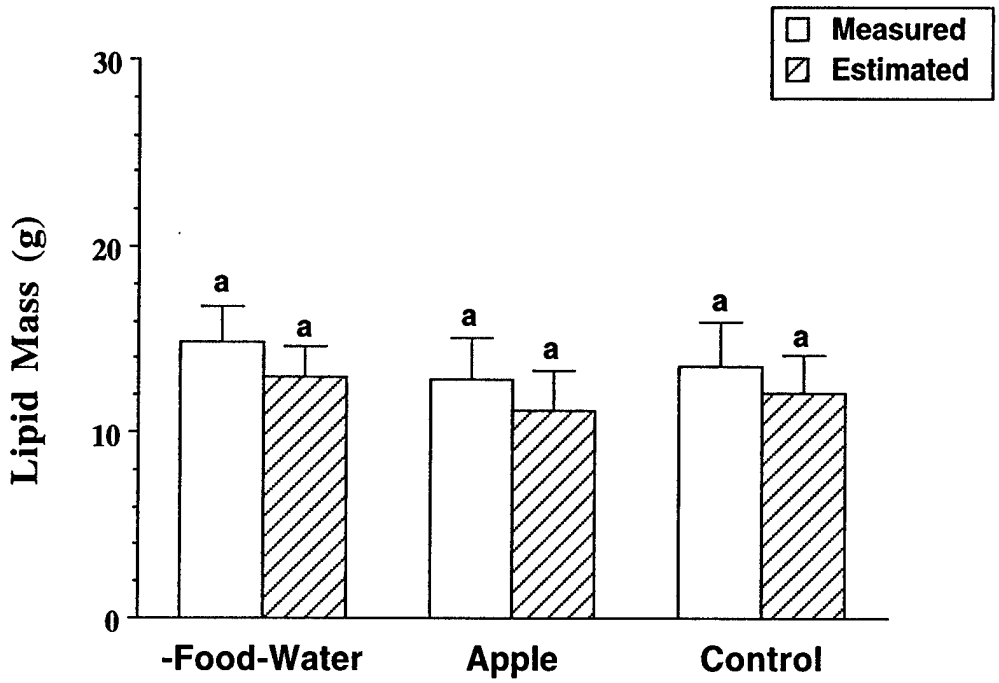


Figure 3.5. Comparison of lipid mass levels estimated by EM-SCAN and lipid mass levels measured by chemical extraction. Values are mean plus/minus one standard error (eeror bars). Different letters indicate significant differences within each group ($p < .05$).

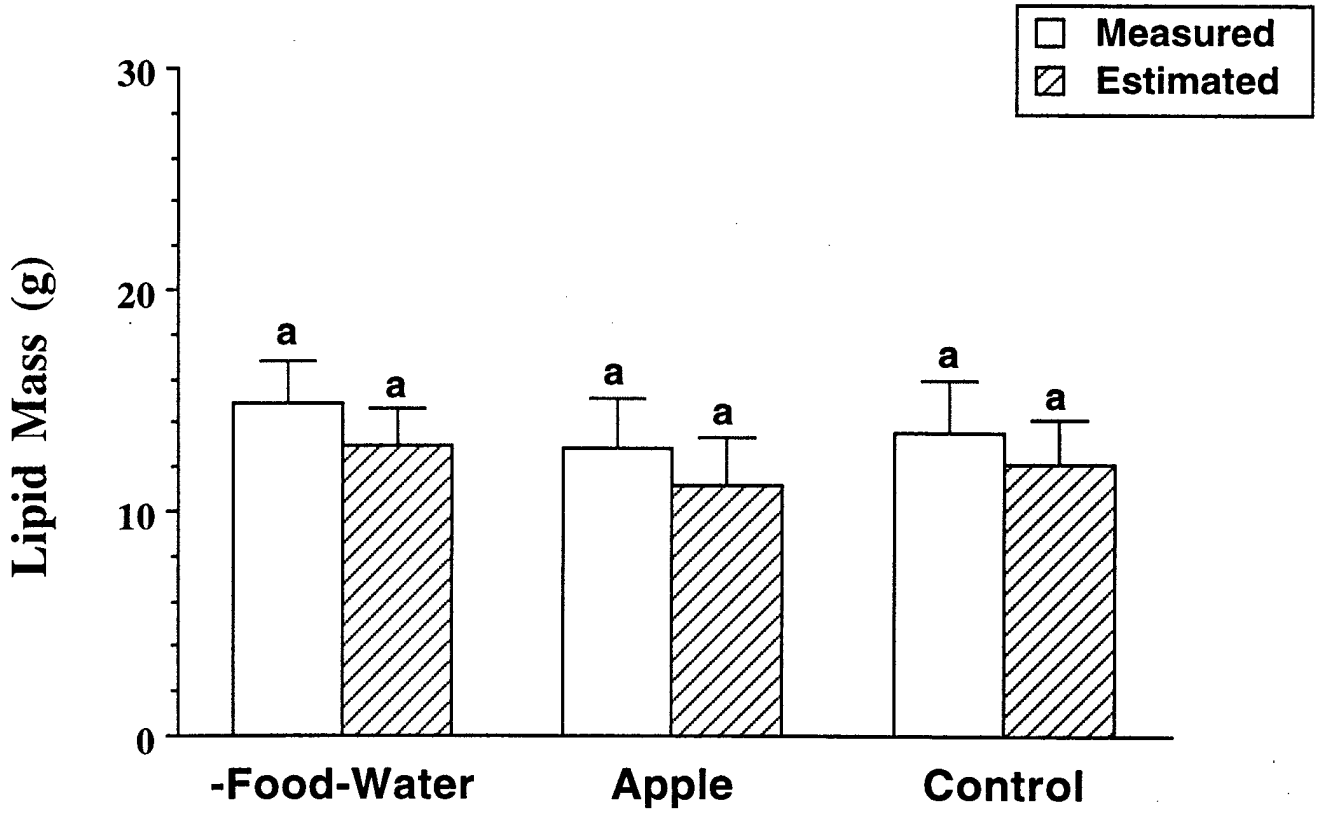
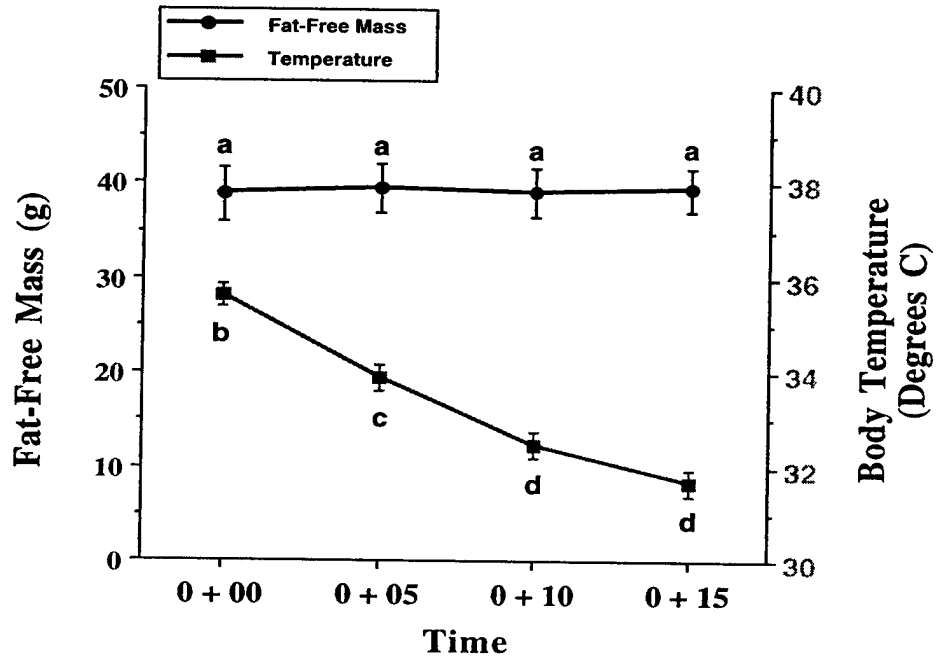
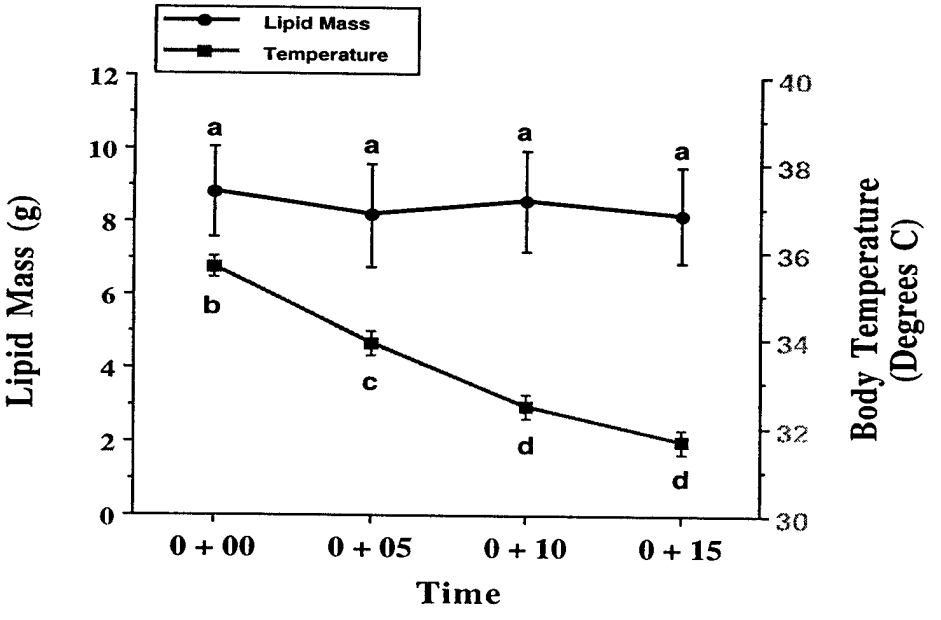


Figure 3.6. Estimates of body composition and measured change in body temperature over 5 minute time intervals for 15 minutes. Values are mean plus/ minus one standard error (error bars). Different letters indicate significant differences between estimates comparing consecutive time intervals ($p < .05$).





Chapter 4

Seasonal patterns in body composition of wild meadow voles (*Microtus pennsylvanicus*) from southeastern Colorado

INTRODUCTION

Prior studies have shown that small, non-hibernating mammals change body mass seasonally, getting smaller in winter and larger in summer (Barbehenn 1955; Brown 1973; Chitty 1952; Fuller et al. 1969; Iverson and Turner 1974; Sealander 1966; Wunder et al. 1977). In addition, most small mammals cease reproduction, reduce growth rates, and exhibit less activity in winter than in other seasons (Barbehenn 1955; Brown 1973; Fuller et al. 1969; Iverson and Turner 1974; Madison 1985; Millar 1981b; Morton & Lewis 1980). Laboratory studies have also shown that photoperiod influences body mass and growth rates in microtines (Dark and Zucker 1983; Dark et al. 1983; Horton 1984a, 1984b; Nagy and Negus 1983; Peterborg 1978; Pistole and Cranford 1982; Pinter 1968; Vaughan et al. 1973).

In addition to body mass change, some general patterns of body composition dynamics are evident from the literature. Body fat in many small, non-hibernating rodents in the field often ranges from 3-8 % of total body mass (Table 4.1). In studies where seasonal change in body composition is reported, percent body fat is usually lower in summer than in winter (Anderson and Rauch 1984; Batzli & Esseks 1992; Cengel et al. 1978; Fehrenbacher and Fleharty 1976; Gyug and Millar 1989; Schreiber and Johnson 1975; Voltura 1997). This is due in part to reproductive females maintaining very low fat

levels in summer (Gyug and Millar 1980; Lochmiller et al. 1983; Millar 1981a; Voltura 1997) and that animals demonstrate seasonal differences in growth and maturation (Barbehenn 1955; Fuller et al. 1969; Brown 1973; Iverson and Turner 1974; Peterborg 1978). However, seasonal change in body fat is not widespread in many non-hibernating mammals. No seasonal trends in lipid levels were found in deer mice (*Peromyscus maniculatus*) (Morton and Lewis 1980; Schreiber and Johnson 1975) or prairie voles (*Microtus ochrogaster*) (Baker 1971; Fleharty et al. 1974; Morton and Lewis 1980).

Prior studies have investigated the use of total body mass and body fat as potential intraspecific nutritional indicators (Iskjaer et al. 1989; Batzli and Esseks 1992). Body size and mass have been used to investigate and explain seasonal patterns and overwinter strategies in small mammals (Chitty 1952; Fuller et al. 1969; Sealander 1966), including meadow voles (*Microtus pennsylvanicus*) (Berbehenn 1955; Brown 1973; Iverson and Turner 1974). In some species, high fat levels are often associated with good physiological condition (Pond 1978; Young 1976). The presence of body fat may be the result of abundant high quality food, reduced energy demands, or storage in preparation for more austere food or energy conditions. However, the use of body fat as a nutritional indicator for small mammals that are relatively lean year-round or show little seasonal variation in body fat may be inappropriate or misleading.

We undertook this study to see whether meadow voles near the southern edge of their North American distribution (Hoffman and Koeppel 1985) show changes in body mass and body composition similar to those reported in more northern areas. Changes in body composition, particularly fat content, often coincide with changing environmental

conditions. Seasonal changes in body composition may also indicate whether fat reserves are an important component in meadow vole overwinter strategy.

MATERIALS AND METHODS

Body composition of wild meadow voles

Using Sherman livetraps (28 x 18 x 13 cm), meadow voles were trapped monthly from three mixed-grass riparian fields (500 m x 300 m each) at the United States Air Force Academy (USAFA), El Paso County, Colorado. We confirmed species identity by periodically examining skulls of animals that died during the study. The three trapping locations are bisected by Lehman's Creek, a perennial stream, and have been undisturbed since the late 1950's. All sites have the dense vegetative structure necessary to support a high vole population (Taitt & Krebs 1985; Peles & Barrett 1996) and are characterized by dense grass and sedge cover. The prevailing vegetation within these sites as described by Ripley (1994) includes smooth brome (*Bromus inermis*), cheatgrass (*Broma tectorum*), Kentucky bluegrass (*Poa pratensis*), thistle species (*Carduus nutans* and *Cirsium arvense*), and snowberry (*Symphoricarpos occidentalis*). In addition, trapping sites included a mixed composition of narrow- and broad-leaved cattails (*Typha angustigfolia* and *T. latifolia*), sedges (*Carex nabrascensis, Eleocharis palustris*, and *Schoenoplectus lacustris*), and rushes (*Juncus arcticus*). The surrounding tall and mixed grass communities are dominated by sandreed (*Calamovilfa longifolia*), big blue stem (*Andropogon gerardii*), blue gramma (*Bouteloua gracilis*), little blue stem (*Schizachyrium*

scoparium), and needlegrass (*Stipa comata)*. One trapping transect was placed within each trap zone approximately 10 m from Lehman's Creek. Each transect consisted of 40 traps, with individual traps placed approximately 5 m apart. Traps were placed beneath the prevailing grass overstory, either on or near visible vole runways. Preference was given to runways which contained fresh grass clippings, indicative of active areas. A four day trapping session was conducted each month from June 1996 through December 1997. During all months, traps were set approximately 1 h before dusk and checked within 1 h of sunrise. From September through April, we also trapped during daylight hours, with traps checked every two hours.

When a vole was found in a trap, it was given an apple slice (approximately 10 g) and piece of lab chow (Lab Diet 5001, PMI Feeds) to assist in rehydration and provide gut-fill following trap retention. Voles were held within capture traps and transported to a laboratory at the United States Air Force Academy within one hour where measurements of body composition were taken using EM-SCAN ® procedures (Voltura and Wunder 1998). Since a consistent body position and lack of subject movement is critical in ensuring estimation accuracy with EM-SCAN®, voles were anesthetized with methoxyfluorane (Metophane®, Mundelein, IL) and then injected with a mixture of Ketamine ® (15mg/kg) (Fort Dodge Laboratories, Inc., Fort Dodge, IO) and Xylazine ® (3mg/kg) (Vedco, Inc., St. Joseph, MO). Voles were weighed to the nearest 0.01 g (Ohaus E400D) and body length without tail measured to the nearest 1.0 mm by stretching animals out along a 250 cm ruler. Body length without tail was then used to center the vole on the chamber insertion platform and within the EM-SCAN® SA-2 chamber itself.

Seven readings were taken on each animal, the highest and lowest values were omitted, and the remaining five values were averaged (Voltura and Wunder 1998). All body composition measures were completed within 1-2 hours after removal from the field each day.

Each vole was toe-clipped while anesthetized to allow recognition (Rose and Hueston 1978; Korn 1987) and returned to its capture location for release. Since pregnant voles maintain extremely low fat levels (Millar 1981b; Kihlstrom 1972; Voltura 1997), no pregnant females (determined by observation and/or abdominal palpation) were used. Since determining an exact age is difficult in voles (Brown 1973, Didow and Hayward 1969), we classified voles less than 29 g as either juveniles or young subadults and did not include them in analyses.

We noted capture site location and gender for each vole. We measured total body mass (Ohaus E400D) and estimated fat-free mass and lipid mass using EM-SCAN®. We also calculated percent lipid mass and percent fat-free mass by dividing lipid mass and fat-free mass by total body mass.

Statistics

Initial investigations of the effects of capture site location and gender on body composition parameters were completed with ANOVA (SAS, proc glm) (SAS Institute, Inc. 1989). Additional comparisons of monthly variation for all composition parameters were also done with ANOVA. To investigate the effects of season on body composition, data from June 96 to June 97 were used. Multiple comparisons between seasons were

done with ANOVA. In addition, the extent of variation in body composition patterns was also performed using a sine function comparison(SAS, proc glm) (SAS Institute, Inc. 1989). For all comparisons, statistical significance was set at $p < 0.05$.

RESULTS

Captures

During the 18 month study, a total of 8640 trap nights were completed. This total was based on 40 traps per location over four consecutive nights, resulting in a total of 480 trap nights per month. Overall capture success was 7.4 % with a total of 641 vole captures, ranging from a high of 53 captures in April 97 to a low of 5 captures in December 97. Of the 641 captures, 73 voles were released immediately at the trap site since they were obviously juveniles (very small size and mass combined with immature dentition) and an additional 82 voles (which were either less than 29 g or determined to be pregnant) were excluded from the data set. The net result was a total of 486 voles for which we measured body composition.

Over the entire experimental period, we had a total of 102 recaptures. Of these recaptures, 79 were captured twice, 19 captured three times, and 4 voles captured four or more times. Since we were interested in monthly change in body composition of individual animals, voles that were recaptured within a specific monthly trapping session were not included in capture totals nor were they remeasured for body composition. In addition, we returned voles to their capture location and released them immediately after body

composition measures. As a result, voles were often released in close proximity to baited traps and were recaptured on consecutive days numerous times.

Body composition

We found no significant differences by month for any body composition parameter due to capture location or gender. As a result, we pooled capture location and gender data by month for subsequent analyses.

Over the 18 month period (Figure 4.1, 4.2), statistical differences between months were found for total body mass ($F_{18,467} = 10.68$; $p = 0.0001$), lipid mass ($F_{18,467} = 2.18$; $p = 0.0034$), fat-free mass ($F_{18,467} = 12.28$; $p = 0.0001$), percent total body lipid ($F_{18,467} = 4.46$; $p = 0.0001$), and percent fat-free mass ($F_{18,467} = 4.46$; $p = 0.0001$).

To investigate seasonal trends in body composition, we chose a 12 month subset of the entire 18 month study (Figure 4.1, 4.2). By using the entire 18 month data, we would have introduced potential year-to-year variation. We selected the period from June 1996 to May 1997 as our 12 month subset. Four seasonal groups were formed using three consecutive month groupings: Summer (June, July, August); Fall (September, October, November); Winter (December, January, February); and Spring (March, April, May). Each seasonal group included a period of changing photoperiod, associated with a seasonal solstice. Significant differences between seasons were present for total body mass ($F_{3,318} = 12.06$; $p = 0.0001$), lipid mass ($F_{3,318} = 1.94$; $p = 0.0341$), fat-free mass ($F_{3,318} = 13.69$; $p = 0.0001$), percent body fat and percent fat-free mass ($F_{3,318} = 3.99$; $p =$

0.0001) (Figure 4.3, 4.4). For these parameters, significant variation between months within each season was also present, with the exception of lipid mass.

During all seasons, lipid mass levels were less than 3 g, but levels were significantly lower in summer (2.10 ± 0.22 g) than in any other season (Figure 4.3). The highest total body mass was present in spring (46.13 ± 1.21 g) with the lowest body mass in winter (37.05 ± 0.71) (Figure 4.3). Since voles in the field are extremely lean year round, the largest component of body composition is fat-free tissue. Thus, fat-free mass levels mirrored total body mass patterns with high lean mass in spring (43.40 ± 0.62 g) and the lowest lean mass levels in winter (34.30 ± 0.66 g) (Figure 4.3). Percent body fat also varied by season with significantly higher body fat in winter (7.37 ± 0.35 %), than in summer (4.84 ± 0.46 %) (Figure 4.4). Significant differences in percent fat-free were also found with summer highs of 95.16 ± 0.46 % and winter lows of 92.63 ± 0.35 % (Figure 4.4).

DISCUSSION

Body composition and seasonal effects

Overall, meadow voles in the field have very low levels of lipid mass throughout the year (< 3 g), with small monthly variation (Figure 4.1, 4.3). Lipid mass ranged from a high of 2.74 ± 0.15 g in winter to a low of 2.10 ± 0.22 g in summer, with about 2.7 g in the fall and spring (Figure 4.3). When comparing lipid mass between seasons, significant differences between summer and all other seasons were found (Figure 4.3). Although

these results are statistically different, we believe such small differences in lipid mass between seasons (maximum of 0.6 g) may not be biologically relevant but that seasonal differences in percent body fat may be more important ecologically. Lochmiller et al. (1983) and Voltura (1997) found identical differences (0.6 g) between seasons in pine voles (*M. pinetorum*) and prairie voles (*M. ochrogaster*) respectively, whereas Anderson and Rauch (1984) reported seasonal effects in lipid mass with July values of 0.6 g and January fat levels of 1.3 g in meadow voles. In all these studies, lipid mass levels were very low but the authors conclude that this difference reflects seasonal change We also found seasonal differences in lipid mass levels but we question its biological importance because lipid mass levels are between 2 and 3 g year-round.

We suggest that seasonal differences in percent body fat, not lipid mass, may be more important in survival situations. At levels below 3 % body fat, lipids are thought to be primarily structural and unavailable during reproduction or energy crises (Robbins 1983; Rock and Williams 1979), whereas higher percent body fat may lead to increased survival time during energy crises. In our 12 month subset, total body mass ranged from 43.62 ± 1.21 g in summer to 37.05 ± 0.71 g in winter, a difference of over 6 g (Figure 4.4). During the same time period, voles maintained between 2 and 3 g of lipid mass regardless of season (Figure 4.3). The resulting percent body fat ranged from 7.37 ± 0.35 % in winter to 4.84 ± 0.46 % in summer, a significant difference between seasons (Figure 4.3). In a model of fasting endurance where fat is catabolized to the 3 % body fat level, Voltura (1996) suggests that a 40 g animal with 4 % body fat would survive 4.5 hours catabolizing body fat and lean tissue at 5° C. In contrast, by increasing percent body fat

from 4 % to 8 %, a 40 g animal can achieve a 22.5 hour survival period. In our study, percent body fat rose from 4 % in summer to over 7 % in winter. Although higher percent body fat in winter was due to decreasing body mass or reduced growth, and not increases in lipid mass, these changes in body composition could potentially increase survival time during energy crises if the Voltura model truly represents reality in the field. Whether voles encounter conditions where they must survive from 4 - 23 hours without food is also unclear. In addition, when ambient temperature is increased from 5° C to 15° C through nesting or huddling with conspecifics (demonstrated by *Microtus*; Wolf 1985), the 40 g animal can increase its survival time from 4.5 hours to 8.5 hours (Voltura 1996).

The fact that voles have low lipid mass levels year-round, yet have lower body mass and higher percent body fat in winter suggests a potential explanation for meadow vole overwinter strategy. Voles in our study had the lowest fat-free mass and percent fat-free mass levels in the winter (Figure 4.3, 4.4). If the survival strategy of lean animals is not to vary lipid mass levels (energy storing), but to spare energy, then the key to winter survival may be dependent upon becoming relatively small (low body mass) with less fat-free mass. In our study adults reduced body mass and fat-free mass, while subadults lessened growth rates during fall and winter to ensure smaller body mass and fat-free mass. Since fat-free mass is more metabolically active than lipid mass, voles can reduce energy requirements by having less lean tissue. Thus, voles born in fall and late summer will not continue to grow to adult size prior to winter. Since voles maintain low lipid mass levels, further growth to adult size and body mass would necessitate increased lean tissue and result in higher daily energy requirements. However, during winter, voles

change body composition due to lowering of body mass which decreases overall energy requirements (Voltura 1996). In our study, body fat increased to 7% in winter, the result of stable lipid mass and decreasing body mass. Thus, voles have greater fat reserves in winter than in summer and may provide additional survival time as suggested by the Voltura (1996) model. However, we still question the reliance on lipid as a primary component in the overwinter strategy on meadow voles. Since voles are lean year-round, voles may be unable to deposit large amounts of fat in the field because they are limited by the quality of food or by the costs of thermoregulation and the costs of acquiring and maintaining fat deposits are greater than the potential benefits accrued. As a result, we conclude that body fat plays an insignificant role in meadow voles and is not an appropriate nutritional index, consistent with the suggestion of Batzli and Esseks (1992).

In our study, there was a significant difference in both body mass and fat-free mass between seasons. Whether this difference is due to the effect of individuals changing mass or changing population structure is not clear. Within a population, seasonal variation in body mass has been attributed to several processes which include: 1) the death of large animals; 2) cessation of growth in young animals; and/or 3) a loss of weight in the older animals that survive (Berbehenn 1955; Brown 1973; Chitty 1952; Fuller et al. 1969; Iverson and Turner 1974; Keller and Krebs 1970; Sealander 1966). In addition, previous studies have suggested that adult meadow voles decrease mass and subadults reduce growth (Berbehenn 1955; Brown 1973; Iverson and Turner 1974; Pistole and Cranford 1982) in winter to cope with increasing energy demands. Using data for voles recaptured in our study, changes in body composition over the winter months support all three

potential causes. As our trapping data show, the mean body mass over the winter months of December, January, and February was significantly lower than in any other season (Figure 4.3). However, the range in body mass for voles captured during winter (20 - 60 g) did not differ from any other season. In summer, voles typically grow at the rate of 0.2 to 0.5 g/day (Berbehenn 1955) which equates to an increase of over 18 g in a three month period. As seen in figure 4.5, four subadult voles (30-40 g) which were recaptured either didn't grow at all or slightly increased body and fat-free mass around 1.5 g/mo during the winter months. Similar reductions in the growth rate of subadults during winter have been previously reported in microtines (Berbehenn 1955; Brown 1973; Iverson and Turner 1974; Horton 1984a, 1984b; Peterborg 1978; Pistole and Cranford 1982; Wunder et al. 1977). Animals born in the late summer stop growing in late fall, maintain their mass and linear dimensions throughout the winter, and resume growth again in the spring (Berbehenn 1955; Brown 1973). Although age was not confirmed in our study, we believe that many voles captured during the winter months were most likely born late in summer or fall, grew to a subadult level (30-40 g), and maintained a smaller body mass over winter. Once conditions improved in spring, they begin to increase body and fat-free mass. We observed a loss in body mass in adult voles with higher body mass (50 - 60 g) prior to winter. But mass losses were not as large as reported in some studies (Gyug and Millar 1989; Mallory et al. 1989; Millar 1981a). In our study, voles we classified as adults in late summer or fall lost less than 7 g of body and fat-free mass (Figure 4.6) during winter. Brown (1973) showed that for a vole born in May, body mass would reach 40 g by August, then decrease to 30 g in fall. Over the winter, such voles maintain body mass

at 30 g levels until the onset of spring. In the Iverson and Turner (1974) study, the mean body mass loss over winter for 14 females to be 45.5 % (49 g in fall down to 26 g in late winter), with a mean loss in 4 males being 28.6 % (41 g down to 29 g).

Our results suggest a latitudinal influence on seasonal effects on body mass in non-hibernating small mammals. Southeastern Colorado represents the southern limit to the distribution of meadow voles in North America (Hoffman and Koeppel 1985). Seasonal changes in thermal stress may be less at lower latitude and photoperiods clearly change less seasonally at lower latitudes. Thus, the smaller body composition responses we observed in this study, compared to the results shown for animals in more northern areas, may be due to less severe environmental conditions in our study location. Therefore, as small mammals inhabit more southern habitat, the expected changes in body composition associated with seasonal change may be reduced.

In our 18 month study, we did not have a single recapture more than six months apart. If this six month pattern reflects the lifespan of voles in this habitat, then many voles born in early spring and summer (April through June) will not survive to the following winter. Thus, we feel that the resident population in winter is comprised primarily of cohorts, born from August through September, which reach and maintain a smaller body mass through winter, combined with a smaller number of mature adults who overwinter by decreasing body mass to some extent. The increase in body mass observed the following spring and summer is then due to the growth of the subadults to adult size and increased fat-free mass in the adult animals.

Body composition pattern (qualitative)

We also wanted to qualitatively describe the general pattern displayed by each body composition parameter over the 12 month period. In order to describe the resulting pattern, we adjusted the appropriate period, phase, and amplitude of the sine function to parsimoniously match the general pattern evident in our data. Thus, we could maximize the variation in the pattern explained by the sine function and provide a qualitative description of the general pattern. The sine model explained 76 % of the month-to-month variation in body mass and 79 % of the monthly variation in fat-free mass (Figure 4.7). In addition, the sine model accounted for 22.4 % of the total variation in body mass and 25.7 % of the total variation in fat-free mass. After accounting for the total variation explained by the sine function, an additional 7.0 % of the total variation in body mass and 6.4 % of the total variation in fat-free mass were explained by month. The remaining 70.6 % of the variation in body mass and 67.9 % of the variation in fat-free mass was due to individual variation of voles within each month.

The one year pattern for seasonal body mass in our study was similar to those reported for meadow voles by Brown (1973) and Iverson and Turner (1974). Although unreported in these studies, the pattern for fat-free mass, the largest body composition component, closely mirrored the pattern for body mass. The general pattern for both body mass and fat-free mass has a shape similar to a sine wave, with summer highs and winter lows (Figure 4.7). Since body mass ranged from 29 g to greater than 56 g during all seasons, there exists a high level of individual body mass and fat-free mass variation both within months and seasons. In contrast, the lipid mass pattern could not be described by

the sine function. Lipid mass levels did not vary more than 1 g throughout the year, with minimal variation between seasons or months. Since these patterns reflect only one year of data, their application to predict cycles and trends is limited. Data illustrating changes in pattern amplitude are necessary to be predictive. However, our results demonstrate a "qualitative" pattern in body mass and fat-free mass that allows for a better understanding of body composition dynamics within the species.

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Table 4.1. Fat content of wild, small mammals. Reported as percent body fat, (s) = seasonal differences, (ns) = no seasonal differences

Species	Fat Content	Reference
Meadow vole (*Microtus pennsylvanicus*)	3 - 7 (s)	1
	3 - 8	11
Pine vole (*Microtus pinetorum*)	5 - 7 (ns)	7
Prairie vole (*Microtus ochrogaster*)	4 - 5 (ns)	8
	3 - 5 (s)	10
	4 - 6 (ns)	2
Red-backed voles (*Clethrionomys gapperi*)	3 - 5 (s)	1
Brown lemming (*Lemmus sibiricus*)	3 - 5 (s)	3
Deer mouse (*Peromyscus maniculatus*)	3 - 5 (s)	9
	5 - 7 (ns)	5, 8
White-footed mouse (*Peromyscus leucopus*)	5 - 8 (s)	6
Harvest Mouse (*Reithrodontomys megalotis*)	3 - 5 (ns)	9
	6 - 7 (s)	5
Grasshopper mouse (*Onychomys leucogaster*)	4 - 6 (ns)	9
Cotton rat (*Sigmodon hispidus*)	5 - 10 (s)	5
Plains pocket gopher (*Geomys bursarius*)	4 - 8 (s)	4
Yellow-faced pocket gopher (*Pappageomys castanops*)	4 - 9 (s)	4

1. Anderson and Rauch 1984
2. Baker 1971
3. Batzli and Esseks 1992
4. Fehrenbacher and Fleharty 1976
5. Fleharty et al. 1973
6. Lynch 1973
7. Lochmiller et al. 1983
8. Morton and Lewis 1980
9. Schreiber and Johnson 1975
10. Voltura 1997
11. This study

Figure 4.1. Summary of body composition of adult wild voles captured from June 1996 through December 1997. Pregnant females and voles less than 29 g were removed. Values reported are mean values pooled from individuals within each month.

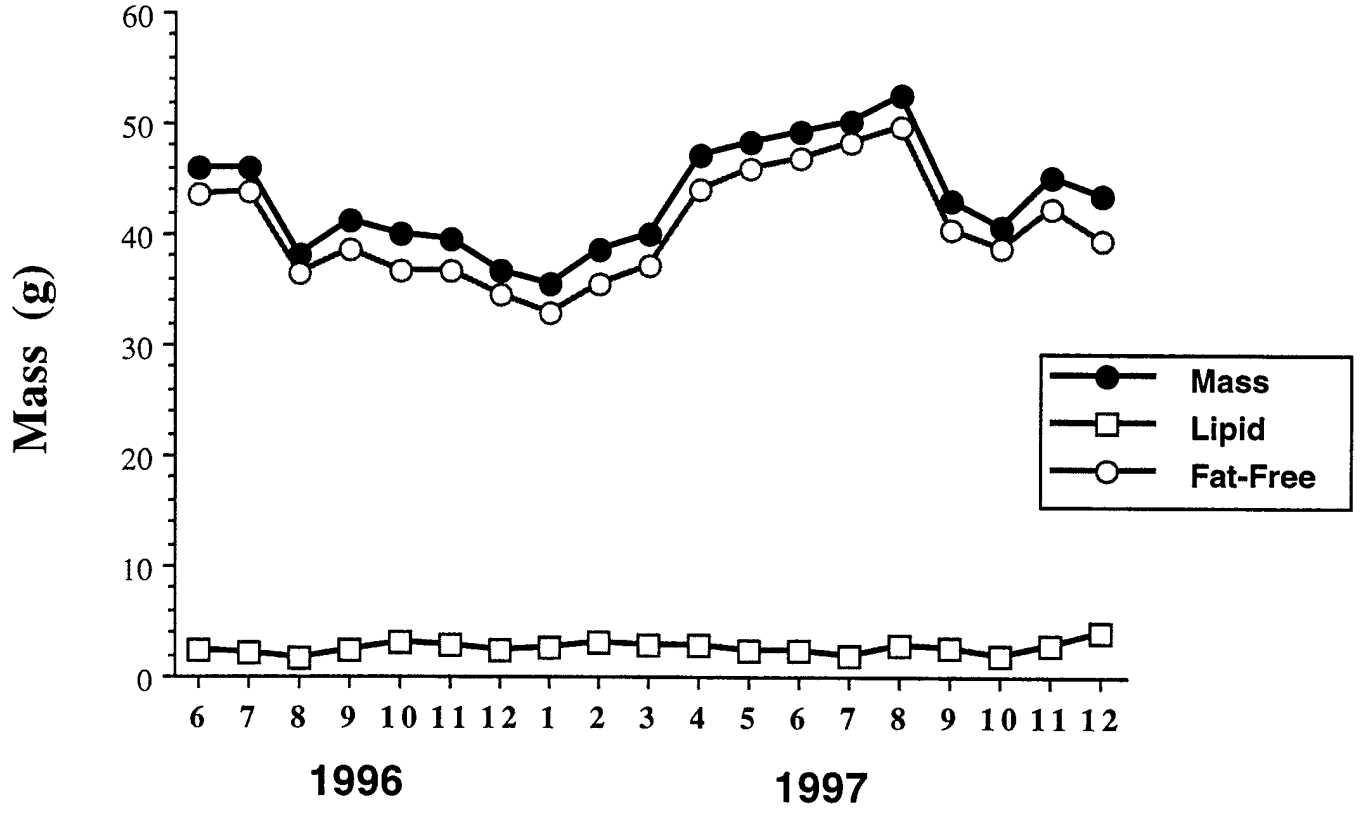


Figure 4.2. Summary of percent lipid and percent fat-free mass for adult wild voles captured from June 1996 through December 1997. Pregnant females and voles less than 29 g were removed. Values reported are mean values pooled from individuals within each month.

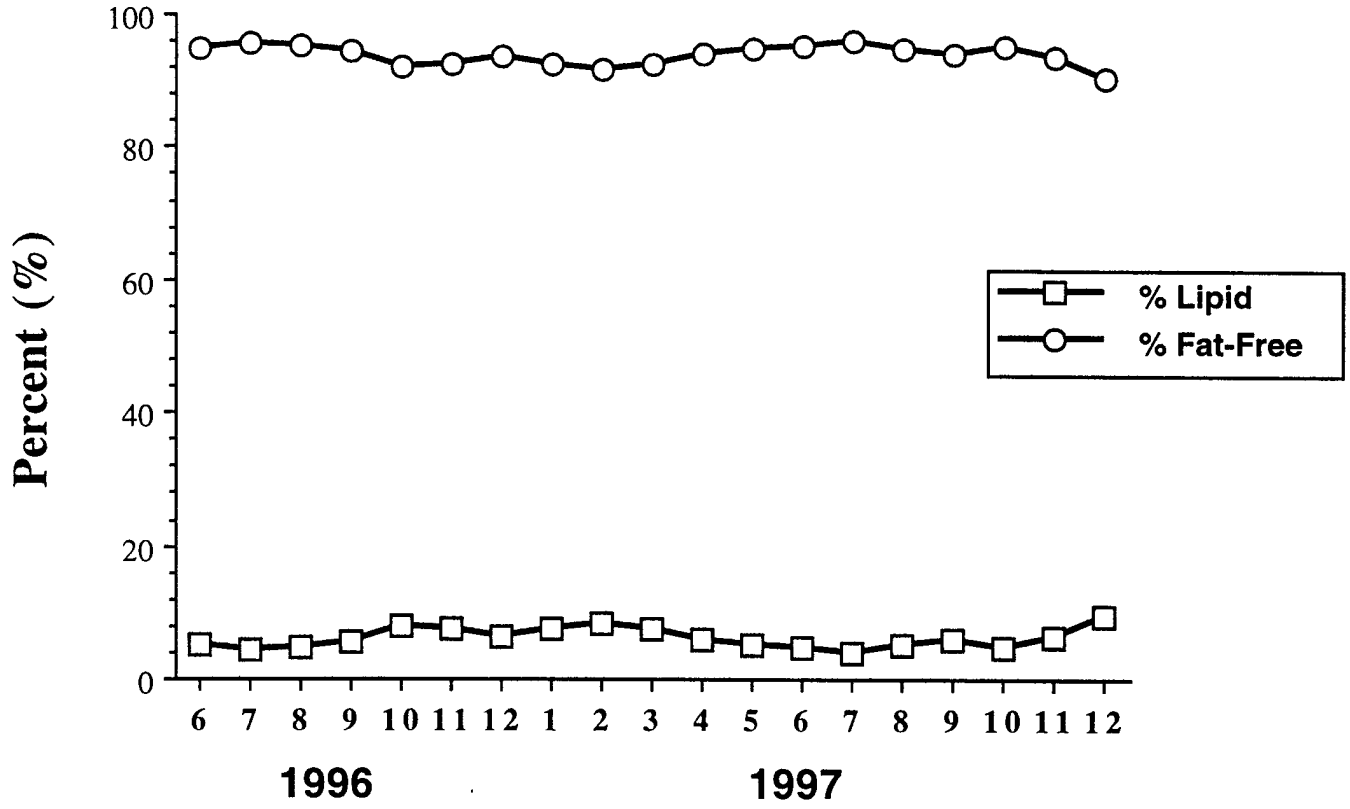


Figure 4.3. Comparison of body composition between seasons. Values are mean plus/minus one standard error (error bar). Different letters indicate significant differences between seasons ($p < .05$). Summer (n = 48), Spring (n = 126), Winter (n = 78), Fall (n = 78).

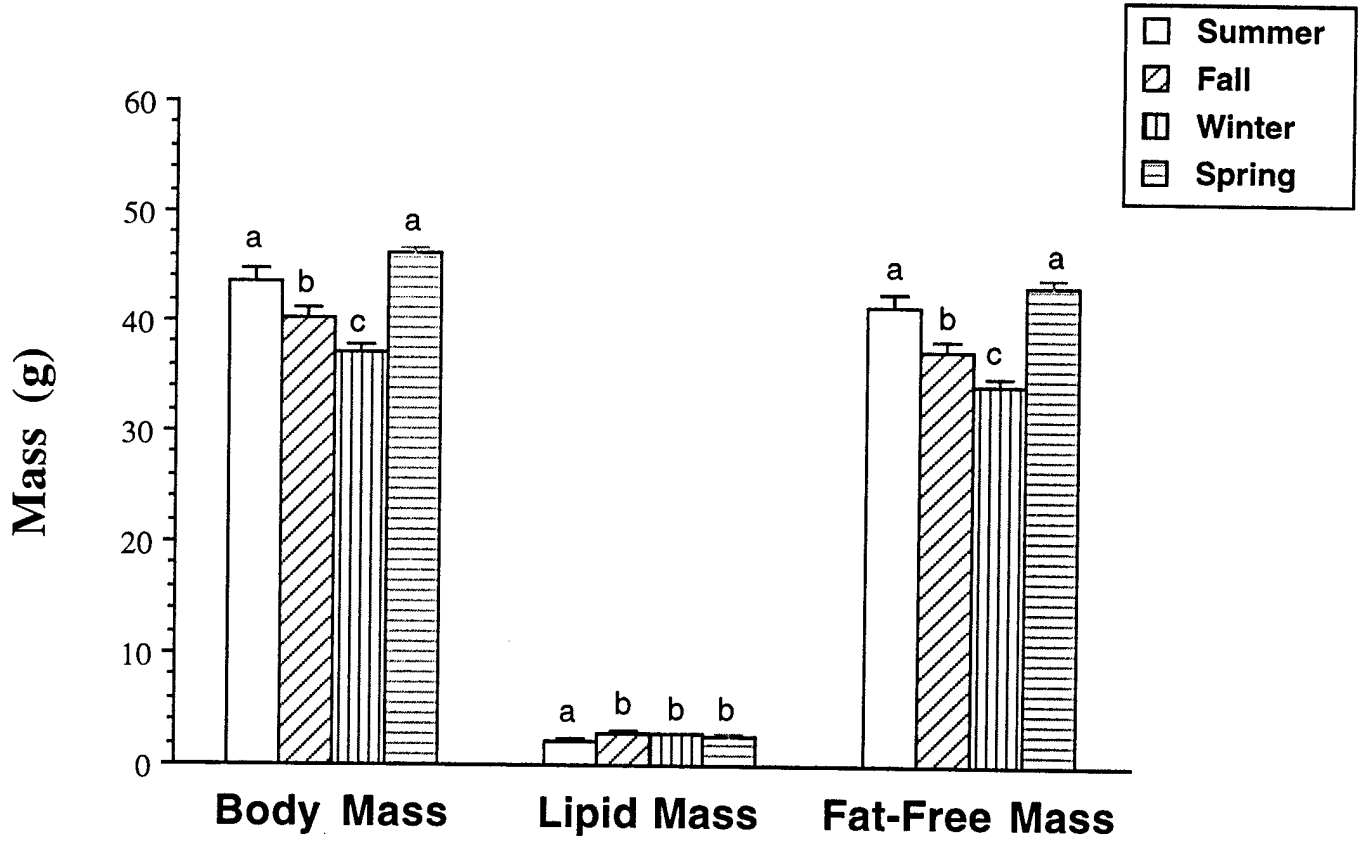


Figure 4.4. Comparison of percent total body fat (A) and percent fat-free mass (B) by season. Values are mean plus/minus one standard error (error bars). Number indicates number of voles per season. Different letters indicate significant differences between seasons ($p < .05$).

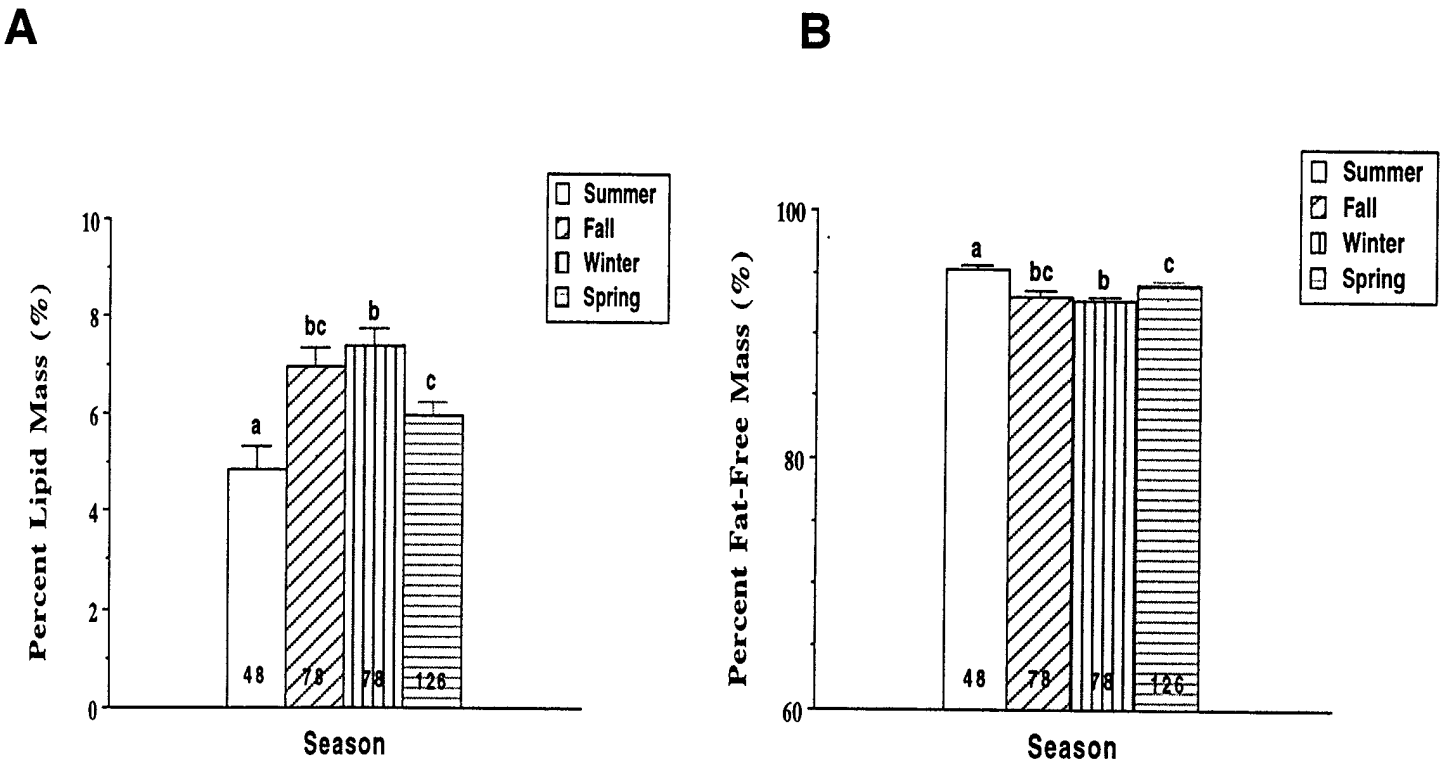


Figure 4.5. Summary of body composition for subadult voles captured over the winter period.

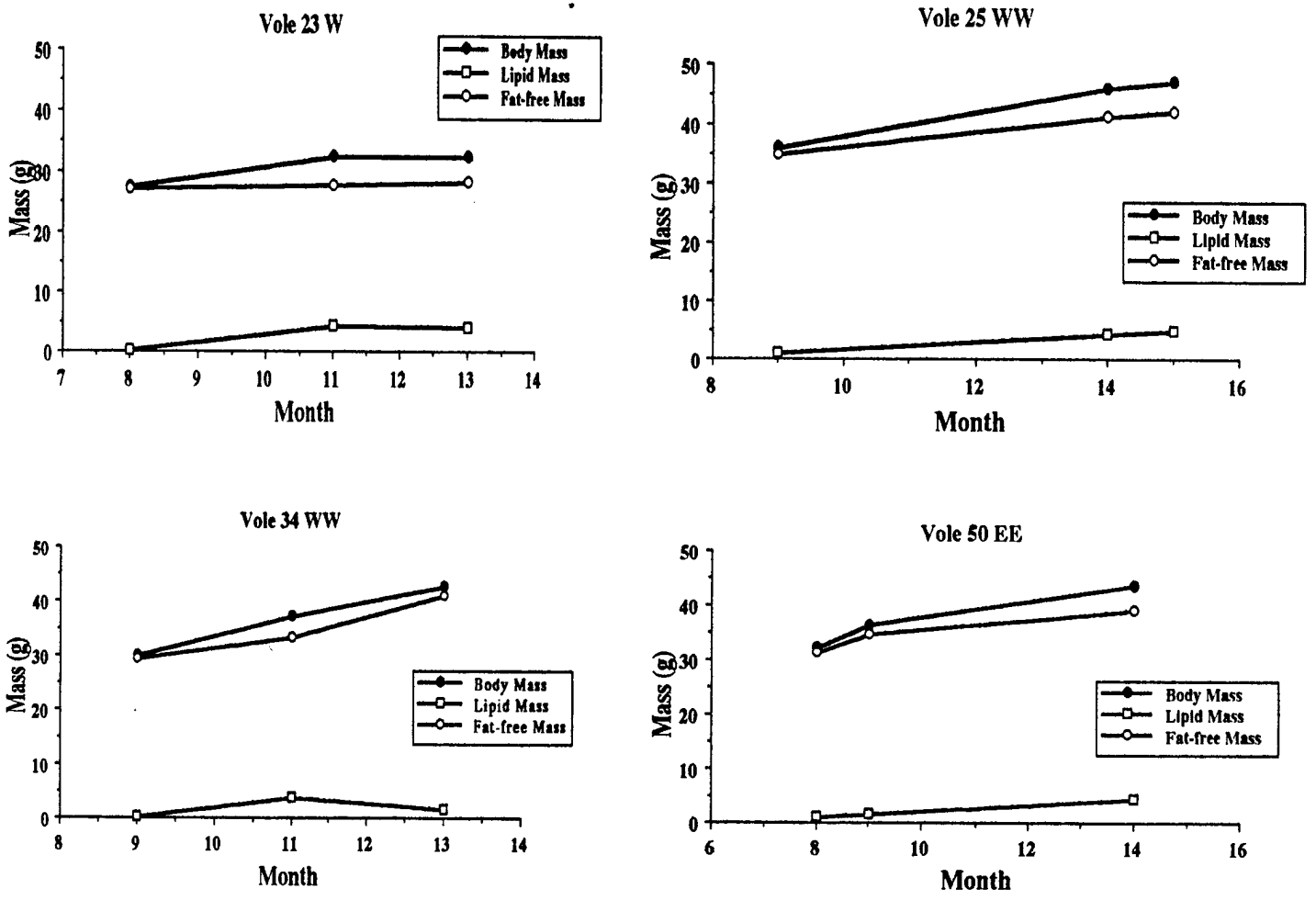


Figure 4.6 Summary of body composition for adult voles captured over the winter period.

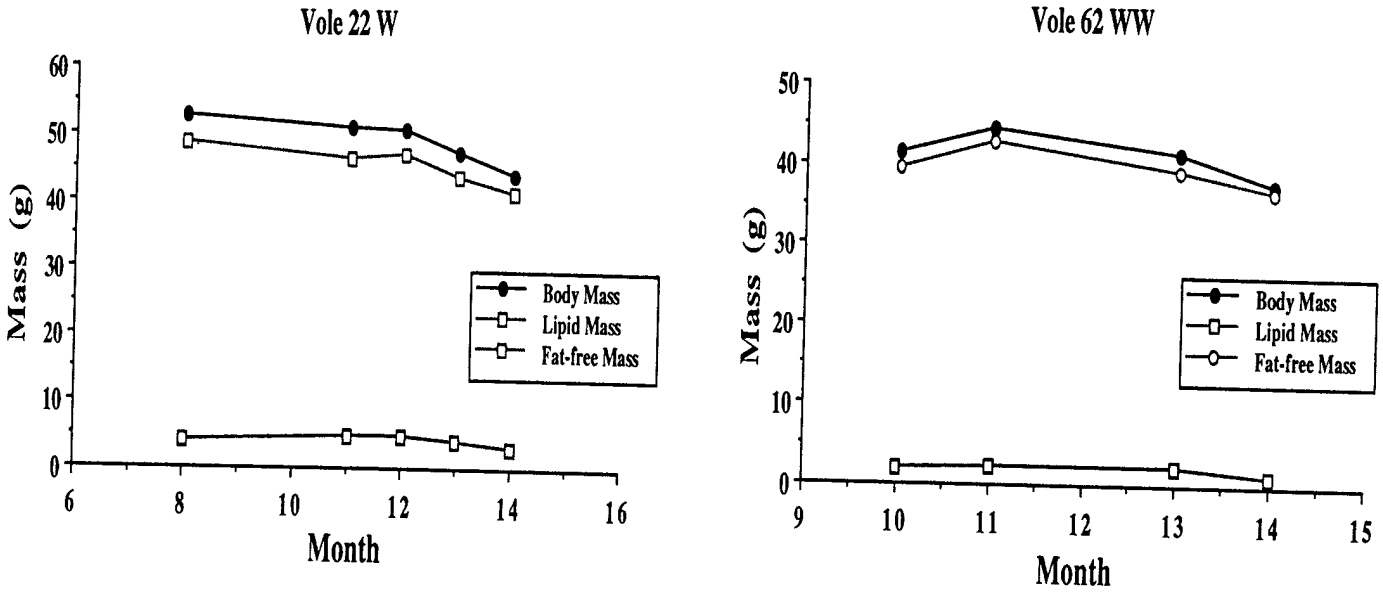
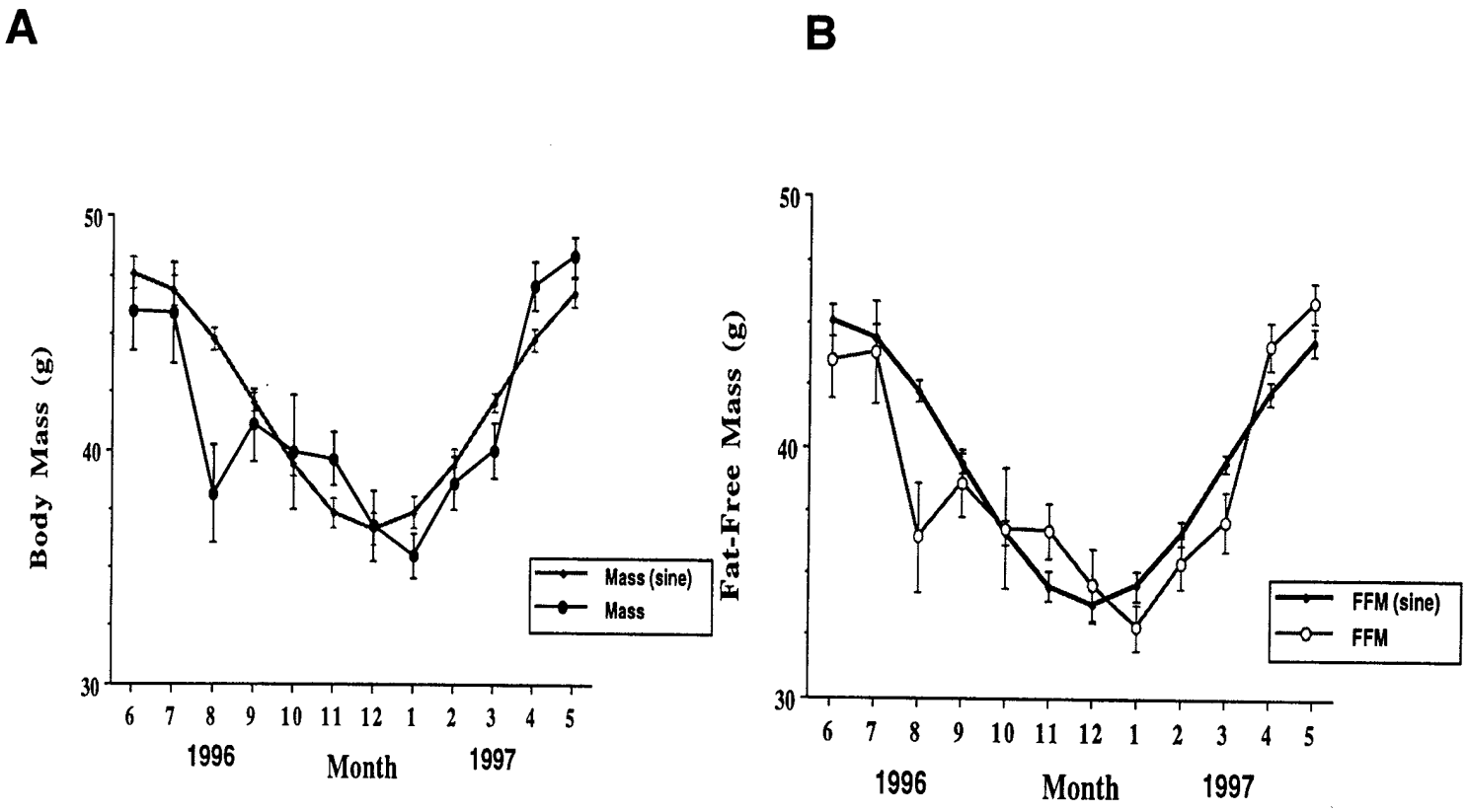


Figure 4.7. Comparison of predictive sine function to total body mass (A) and fat-free mass (B) for period from June 1996 to May 1997. Values are mean plus/minus one standard error (error bar).



Chapter 5

Effects of food quality on body composition of wild meadow voles (*Microtus pennsylvanicus*) maintained under laboratory conditions

INTRODUCTION

Initially suggested by Dehnel (1949) for shrews, body mass has been reported to show seasonal variation in small mammals (Berbehenn 1955; Brown 1973; Dehnel 1949; Fuller et al. 1969; Iverson and Turner 1974; Sealander 1966). In these studies, the mean body mass of individuals composing populations of north-temperate small mammals is lower in winter and early spring than in summer and fall. In addition, the effect of photoperiod and ambient temperature on body mass dynamics in the lab has been demonstrated for collared lemmings (*Dicrostonyx groenlandicus*) (Nagy 1983; Nagy and Negus 1983; Nagy et al. 1994), montane voles (*Microtus montanus*) (Pinter 1968; Peterborg 1978; Vaughan et al. 1973), and meadow voles (*Microtus pennsylvanicus*) (Dark and Zucker 1983; Dark et al. 1983; Pistole and Cranford 1982). In these studies, voles decreased body mass with decreasing photoperiod, but the authors did not address changes in body composition.

In the wild, fat levels of many small mammals are quite low, often ranging from 3 to 8 % of total body mass (Anderson and Rauch 1984; Batzli and Esseks 1992; Baker 1971; Didow and Hayward 1969; Fehrenbacher and Fleharty 1976; Fleharty et al. 1973; Lochmiller et al. 1983; Lynch 1973; Morton and Lewis 1980; Nestler 1996; Rock and Williams 1979; Sawicka-Kapista 1969; Schreiber and Johnson 1975; Voltura 1996, 1997) (Table 5.1). Levels of body fat may vary throughout the year with seasonal effects

reported for some species. However, seasonal change in body composition is not widespread among non-hibernating small mammals. No seasonal trends in lipid levels were found in deer mice (*Peromyscus maniculatus*) (Morton and Lewis 1980; Schreiber and Johnson 1975) or prairie voles (*Microtus ochrogaster*) (Baker 1971; Fleharty et al. 1974; Morton and Lewis 1980). In an 18 month field study, we did not find seasonal variation of body fat in meadow voles (*Microtus pennsylvanicus*), rather they showed relatively constant lipid mass throughout the year (Chapter 4). Potential causes for such leanness in the wild may be due to high metabolic demand, low diet quality and/or availability, or an active lifestyle.

Numerous studies of small mammals have proposed using body fat as an index of nutritional condition (Cranford 1978; Franzmann 1985; Galster and Morrison 1976). For many small, non-hibernating mammals, the adaptive significance of fat deposits lies in their role as supplemental or emergency caloric sources (Pond 1978, 1981; Millar 1981). Since fat deposits are directly related to overall energy balance of an animal, a lean body condition may imply a diminished nutritional condition. However, a lean body could also suggest a low need for future energy reserves (Millar and Hickling 1990), or it could be due to activity (Batzli and Esseks 1992) or diet (Cengel et al. 1978). Thus, low fat body levels should not necessarily be interpreted as maladaptive (Morton and Lewis, 1980) and the interpretation of temporal changes in body fat is not always clear. As suggested by Batzli and Esseks (1992), body fat may not be an appropriate nutritional index for many small mammal species. In lean species, adaptive strategies for survival may be directed more toward energy sparing than energy storing.

Whether field-caught (Batzli and Esseks 1992; Voltura 1996, 1997) or lab-reared (Donald et al. 1980; Ferns and Adams 1974; Holleman and Dieterich 1978; Sawicka-Kapusta 1970, 1974; Voltura and Wunder 1998), small mammals held under laboratory conditions will within weeks increase body fat to levels far beyond those seen in the field. In these studies, body fat often exceeds 10 % of body mass within 30 days when animals are kept in the laboratory. The potential causes for such body composition change may be due to the effects of confinement which reduces activity, unnatural, highly digestible diets, stable environmental conditions, or any combination thereof.

Thus, we undertook this study to evaluate the effects of food quality on the body composition of meadow voles removed from the field and kept under stable environmental conditions. Using non-invasive and repeatable measures of individual animals over time, we investigated both the extent and rate of fattening. In an additional series of experiments, we also varied food quality (fat content and digestible energy), as well as temperature and photoperiod to evaluate the effects of diet quality on changes in body composition.

MATERIALS AND METHODS

Experiment 1. *Body composition of wild meadow voles held under laboratory conditions and fed lab chow*

Meadow voles were trapped using Sherman livetraps (28 x 18 x 13 cm) from local populations in three mixed-grass riparian fields at the United States Air Force Academy, El Paso County, Colorado. Trapping was conducted in January, April, and June of 1997.

These trapping periods were chosen to represent different seasons. A September 1997 trapping session was also completed, with the group to be included as part of this experiment. However, due to the results we obtained from the first three groups, we exposed the September group to a different experimental manipulation as explained later in experiment 3. Data from the April group were also included as part of an additional experiment described in experiment 2. Lastly, we included data from the June group as part of experiment 3.

During each trapping session, we collected at least twelve adult voles ensuring an equal number of males and females for each group. Since pregnant voles maintain extremely low fat levels (Millar 1981), all pregnant females (determined by observation and/or abdominal palpation) were omitted. In addition, voles less than 29 g total body mass were not used. Since voles less than 29 g are most likely either juveniles or young subadults, it would be difficult to determine whether changes in body composition were due to experimental conditions or growth. Within the January group, five voles less than 29 g were used in the experiment due to low trapping success. For all trapping sessions, traps were set approximately 1 h before dusk and checked within 1 h of sunrise. During January and April, trapping also occurred during daylight hours, with traps checked every two hours.

When traps were checked, captured voles were given an apple slice and piece of lab chow (Lab Diet 5001, PMI Feeds) to assist in rehydration and provide gut-fill. Voles were held in capture traps and transported to a laboratory at the United States Air Force Academy to estimate body composition using an EM-SCAN® device as in Voltura and

Wunder (1998). All body composition estimates were completed within 2 - 3 hours after traps were checked. Voles were then taken to Colorado State University, Fort Collins, CO, and housed in an environmental chamber at 23° C with natural photoperiod. Voles were housed in individual cages (28 x 18 x 13 cm) with a premeasured amount (approximately 100g) of lab chow (Lab Diet 5001, PMI Feeds) and water provided weekly. Although we started each session with twelve voles (6 male, 6 female), voles which did not survive the entire experimental period were omitted from analyses. This resulted in unequal numbers of voles in each session. We measured total body mass (Ohaus E400D, 0.01 g) and body composition (fat-free mass and lipid mass) weekly for six weeks. Food was replaced weekly after removing orts. We measured weekly food intake by subtracting the orts from the food offered. Individual intake values were summed by group and then divided by seven for daily intake.

Experiment 2. *Food quality effects on body composition in wild meadow voles held under laboratory conditions*

During the April trapping period, we captured an additional six voles resulting in a total of 18 captures. Experimental procedures were identical to those used in experiment 1 with one notable exception: two groups of wild voles were held under identical laboratory conditions but fed different foods. One group (12 voles) was fed lab chow (Lab Diet 5001; PMI Feeds) with the other group (6 voles; 3 males, 3 females) given rabbit chow (Lab Diet 5325, PMI Feeds). The results for the group fed lab chow were gathered from the voles described in experiment 1. Rabbit chow most closely approximates the nutritive value of the preferred natural diet of microtines (Batzli and

Esseks 1992) with a fat content of less than 2 % and a higher crude fiber content (22.5 %) (PMI Feeds analysis) than lab chow. The actual composition of each diet (as reported by the manufacturer) is provided for comparison in Table 5.2. Both diets have been used by investigators to maintain animals in the lab and are considered nutritionally balanced.

Experiment 3. *Effects of dietary fat in food on body composition in wild meadow voles held under laboratory conditions*

For this experiment, we compared the data collected from the June group in experiment 1 to a group of 12 additional voles captured in September. Since the results from January, April and June showed no seasonal effect on changes in body composition when brought into the laboratory, we assumed no difference due to time between groups. The procedures for this experiment were identical to those used in experiment 1 except the voles captured in September were fed a high-fat diet instead of lab chow. Then, the diet was switched to a lab chow diet for three more weeks. The high-fat diet consisted of lab chow with added fat (vegetable oil), resulting in a calculated dietary fat content of approximately 25 %. Lab chow was mixed with vegetable oil (4:2) to form a paste and then pressed into cakes with a quarter pound hamburger press. To reduce fat volatilization, the cakes were baked at a low temperature (100° C) for four hours and then further dried for 24 hours at 50° C to remove as much water as possible. Vegetable oil was selected to maximize available polyunsaturated fats, the most prevalent lipid form in natural vegetation (National Research Council 1964; VanSoest 1994). To ensure nutrient dilution did not occur in the high-fat diet, a proximate analysis (Nahm 1992) of the high-fat diet was completed by the Soil, Water, and Forage Analysis Lab, Colorado State

University, Ft. Collins, CO, and is presented in Table 5.3. After maintenance on the high-fat diet for six weeks, September voles were switched to lab chow (Lab Chow 5001, PMI Feeds) for an additional three weeks to investigate whether lowering fat content would further affect body composition. As previously detailed, the June voles were fed lab chow for six weeks, then fed the high-fat diet for three more weeks. Thus, the order of food presentation was reversed between these groups brought in from the field. We compared body composition patterns between the June and September groups to determine whether varying the fat content of food would affect changes in body composition and whether the order of presentation would affect the response patterns.

Experiment 4. *Effects of natural vegetation on body composition of field-captured meadow voles maintained under laboratory conditions*

In all previous experiments in this study, voles were fed unnatural, highly digestible diets. As a result, we felt it necessary to examine the effects of natural vegetation on body composition. Trapping was done in October 1997 at the United States Air Force Academy using the procedures described in experiment 1. The experimental procedures for this study were the same as in experiment 1 except voles were fed the natural vegetation. Natural vegetation was collected from trap sites by selecting 1 m by 1 m plots along the trapping transect. Within each plot, all vegetation was clipped at ground level and the entire plant (all above-ground vegetative parts) placed in plastic bags and held in a freezer until needed. A proximate analysis (Nahm 1992) of one vegetation sample (500 g wet mass) was performed as described in experiment 3 and results are presented in Table

5.4. Each vole received 50 g of vegetation per day and was fed twice daily (approximately 25 g) at 0800 and 1700. This quantity was selected assuming each vole consumed no more than 15 g dry matter per day as shown in prairie voles by Castle and Wunder (1995), and that the cut vegetation contained approximately 70 % water. We cut the vegetation into 6 cm long pieces to fit into the cage.

Experiment 5. *Effects of environmental conditions and food quality on body composition of meadow voles held under laboratory conditions*

Since both temperature and photoperiod have been reported to affect the body mass of several small mammals (Giser and Heldmaier 1995; Hoffman 1973; Hoffman and Johnson 1985; Horton 1984a, 1984b; Kriegsfeld and Nelson 1996; Lynch and Gendler 1980; Mallory et al. 1981; Nagy 1993; Nagy and Negus 1993; Nagy et al. 1994; Pinter 1968; Peterborg 1978; Rhodes 1989), including meadow voles (Dark and Zucker 1983; Dark et al. 1983; Pistole and Cranford 1982), we designed this experiment to include these factors combined with varying food quality. Thirty-two meadow voles approximately two months of age (50 g) were selected from a breeding colony established at Colorado State University and maintained on a photoperiod of 16L:8D at 23° C. The voles were ranked by body mass. The four heaviest voles were randomly assigned to one of four treatments. The procedure was repeated for the next four animals and continued until all animals were assigned. We selected treatment conditions that we felt were representative of naturally occurring conditions: 1) 16L:8D, 23° C, lab chow (Group D); 2) 16L:8D, 23° C, high-fat diet (Group C); 3) 8L:16, 5° C, lab chow (Group A); and 4) 8L:16, 5° C, high-fat diet (Group B). We linked long day conditions (16L:8D) with warm temperature (23° C) and

short day conditions (8L:16D) with cold temperature (5° C). Although a factorial design with three conditions has numerous potential combinations of factors, we selected conditions which were the most ecologically realistic. For each vole, body composition was measured as in experiment 1. Since we tested the effects of photoperiod and temperature on body composition, we felt that the experimental period should be extended to 10 weeks to allow for possible physiological changes to occur. To minimize handling stress, we used biweekly measures instead of weekly analyses.

During the first week on the experiment, a malfunction in an environmental chamber housing Group D resulted in the mortality of six voles. Therefore, we removed Group D from the analysis and proceeded with an unbalanced design using three groups and eliminated the group with lab chow and long day, warm conditions. Since voles exposed to differing photoperiods had been investigated in experiment 1 with no significant effect on body composition change, we felt comparisons still could be made.

Statistics

For experiments 1, 2, and 4, a repeated measures ANOVA (SAS, proc mixed) (SAS Institute, Inc., 1989) with time (week) as the fixed effect and subject as a random effect was performed for each body composition parameter: total body mass, lipid mass, fat-free mass, percent lipid mass, and percent fat-free mass. To investigate the degree of similarity in body composition between groups in experiment 1, we used a repeated measures ANOVA (SAS proc mixed) with time (week) and seasonal group as fixed effects and subject nested within group as the random effect. In experiments 2, 3, and 5,

comparisons between groups for all body composition parameters were made with repeated measures ANOVA (SAS proc mixed) using time (week) and treatment (environmental condition, food type) as fixed effects and subject nested within treatment as a random effect. For all experiments, a repeated measures ANOVA was also completed for food intake and intake per gram body mass. In addition, a change value for each body composition parameter was calculated to better illustrate the magnitude of change over time. Change values included the change from start to week 2, start to week 4, and start to week 6, and week 6 to week 9 (when appropriate). Change values were calculated by subtracting the initial value for each parameter from the week 2, week 4, or week 6 value respectively, and week 6 from week 9. For all comparisons, statistical significance was set at $p < 0.05$.

RESULTS

Body composition of wild meadow voles held under laboratory conditions and fed lab chow

For all groups, body composition change over time in the laboratory showed that gains in total body mass were mainly due to large increases in lipid mass coupled with small losses in fat-free mass (Figure 5.1, 5.2, 5.3). Within any seasonal group, all body composition parameters demonstrated significant change over time ($p < 0.01$). In all cases, voles reach a plateau in body fat and body mass within three weeks. In addition, the levels reached from week 3 through week 6 were significantly different ($p < 0.01$) from the level at the start of the experiment for each body composition parameter. In the

January group, a gain in total body mass of 6.18 ± 2.18 g was due mainly to an increase in lipid mass (8.84 ± 1.21 g), combined with a loss of fat-free mass (2.95 ± 1.30 g) (Table 5.5). The April capture group gained 8.90 ± 1.79 g in total body mass with increases in lipid mass of 10.29 ± 1.12 g and losses of fat-free mass of 2.50 ± 1.17 g (Table 5.5). Similar change was shown in the June group with an increase in total body mass (7.08 ± 2.97 g) due to lipid mass gains of 10.40 ± 1.85 g and fat-free mass losses of 4.29 ± 2.27 g (Table 5.5).

When voles were removed from the field, the January group had lower total body mass and fat-free mass than the April and June groups ($p < 0.05$). Therefore, further comparisons between seasonal groups used percent lipid mass and percent fat-free mass. No significant time by group interaction for either percent lipid mass or percent fat-free mass ($F_{10,166} = 1.15$; $p = 0.3279$) was present and suggests no seasonal effect on body composition change. In addition, the lack of significant differences for both percent lipid mass and percent fat-free mass between groups ($F_{2,30} = 0.52$; $p = 0.5971$) further supports this conclusion (Figure 5.4). Overall, when meadow voles are removed from the wild and held in the lab with lab chow under stable experimental conditions, they increase total body mass and lipid mass and decrease lean mass regardless of season of capture.

Food quality effects on body composition in wild meadow voles held under laboratory conditions

No significant differences between diet types for any body composition parameter over time were evident. Voles eating rabbit chow gained 6.16 ± 1.13 g of body mass with an increase in lipid mass of 8.26 ± 2.11 g and a loss in fat-free mass of 2.10 ± 2.57 g (Table 5.6). As reported for experiment 1, the April group fed lab chow gained 8.90 ± 1.79 g of total body mass, with increases in lipid mass of 10.29 ± 1.12 g and losses in fat-free mass of 2.50 ± 1.17 g (Table 5.6). Thus, regardless of food type consumed, the body composition pattern over time was similar. Weekly comparisons between groups for each body composition parameter showed no significant differences: total body mass ($F_{1,12}$ = 0.01; p = 0.9054), lipid mass ($F_{1,12}$ = 0.61; p = 0.4499), fat-free mass ($F_{1,12}$ = 0.25; p = 0.6231), percent lipid mass ($F_{1,12}$ = 0.67; p = 0.4293), and percent fat-free mass ($F_{1,12}$ = 0.67; p = 0.4293). When voles ate rabbit chow, the small change in body mass and body fat during the first two weeks probably reflected a lag due to lower diet quality which was eventually overcome via a higher food intake. The large increase in body fat during week 3 with rabbit chow was probably due to the effects of higher intake the preceding two weeks (Figure 5.5, 5.6). Nevertheless, whether eating lab chow or rabbit chow, wild voles increased total body mass, due primarily to gains in body fat (Figure 5.5).

There were significant differences in food intake to effect similar changes in body composition between voles eating different food types ($F_{1,12}$ = 15.59; p = 0.0019), and over time ($F_{3,36}$ = 15.56; p = 0.0001). As expected, during all weeks, food intake was greater for voles eating rabbit chow than lab chow (Figure 5.6). As seen in Table 5.2,

rabbit chow has higher fiber (25 %) and lower gross energy (3.90 Kcal/g) than lab chow (6.0 %, 4.25 Kcal/g). In addition, physiologic fuel value, calculated as the sum of protein, fat, and carbohydrate times 4, 9, and 4 Kcal/g respectively was lower for rabbit chow (2.43 Kcal/g) than lab chow (3.30 Kcal/g). After week 1, both groups decreased intake in the following three weeks (Figure 5.6). This four week period coincides with the greatest change in body composition. From start to week 4, voles eating lab chow gained 8.81 ± 1.46 g of lipid mass with losses of 4.22 ± 1.07 g of fat-free mass. During the same period, voles eating rabbit chow increased lipid mass (6.42 ± 1.22 g) with losses in fat-free mass of 1.83 ± 2.12 g (Table 5.4). Between groups, these changes were not statistically different, although voles eating rabbit chow did gain less lipid and lost less fat-free mass. We suggest that this is due to a higher gut fill in voles eating a higher amount of rabbit chow as compared to lab chow. In both groups, voles changed body composition and increased body mass, but food intake within each group remained relatively constant throughout the period. Since the change in body composition was due to increases in body fat (which has much lower metabolic requirements than lean tissue) and loss of fat-free mass (an additional decrease in energy requirements), a subsequent increased intake within a particular food was not necessary.

Effects of fat levels in food on body composition in wild meadow voles held under laboratory conditions

Results reported for the June group in experiment 1 showed that voles eating lab chow over six weeks increased total body mass (7.08 ± 2.97 g), with gains in lipid mass of 10.40 ± 1.85 g and losses of fat-free mass (4.29 ± 2.27). When the diet was switched after six weeks to a high-fat diet for an additional three weeks, voles gained an added 5.86 ± 0.81 g of total body mass, due to similar increases in lipid mass (2.99 ± 0.55 g) and fat-free mass (2.88 ± 0.57 g) (Figure 5.8; Table 5.7). In September 1997, voles right from the field were given the high-fat diet rather than lab chow. After six weeks on the high-fat diet, voles gained 13.18 ± 0.94 g of lipid mass to a body composition of 26.58 ± 1.18 % body fat by week 6. Over the same time period, total body mass increased 5.74 ± 2.08 g, with losses in fat-free mass of 7.44 ± 1.53 g (Figure 5.9; Table 5.7). When diet was changed to lab chow for three additional weeks, voles lost 8.69 ± 1.22 g in total body mass from the body mass at week 6. During these three weeks, voles lost 6.41 ± 1.81 g of lipid mass and 3.52 ± 1.02 g of fat-free mass from levels at week 6.

When eating a high-fat diet, voles gained more lipid mass and lost more fat-free mass (Table 5.5). Since voles consuming lab chow ate approximately 1.5 g more food per day than those on the high-fat diet, the smaller losses in fat-free mass may be due to gutfill. Overall, our results suggest that meadow voles respond differentially to different levels of dietary fat. The response in body composition varies directly with the level of dietary fat.

For the September group, food intake of the high-fat diet decreased from start to week 6, with no significant differences between weeks. When food was switched to lab chow, a slight increase in intake resulted but the difference was not significant (Table 5.8). In the June group, voles eating lab chow also decreased intake over the six week period. However, within this group, comparisons between consecutive weeks showed a significant difference in intake from week 2 to week 3 only (Table 5.8). When provided the high-fat diet, intake further decreased, reflecting the higher caloric value per gram of food due to increased fat in the high-fat diet.

Effects of natural vegetation on body composition of field-captured meadow voles maintained under laboratory conditions

We are unable to address the effects of natural vegetation on body composition due to high vole mortality. Within one week after capture, eight of twelve voles had died without any prior indication of adverse health. Voles which died had lost around 20 % total body mass, but seemed to be consuming both food and water. Perhaps, the vegetation cleared from trapping areas and fed to these voles was not what they actually consume in the wild. In addition, vegetation provided may not have contained essential nutrients and minerals necessary to maintain mass (Batzli 1985; Christian 1989). However, the vegetation we collected and provided to the voles was representative of the habitat from which the voles were collected. Thus, we felt voles would eat this vegetation when provided.

Effects of environmental conditions and food quality on body composition of meadow voles held under laboratory conditions

There was a significant interaction between group and time for total body mass ($F_{16,137} = 4.50$; $p = 0.0001$), lipid mass ($F_{16,137} = 4.50$; $p = 0.0001$), fat-free mass ($F_{16,137} = 3.47$; $p = 0.0001$), percent lipid mass ($F_{16,137} = 2.51$; $p = 0.0001$), and percent fat-free mass ($F_{16,137} = 2.51$; $p = 0.0001$). Over the 10 week test, differential changes in lipid mass and body mass over time due to treatment were found (Figure 5.10, Figure 5.11), Although changes in fat-free mass were present within a treatment, no significant differences between treatments were evident (Figure 5.12). The largest gains in total body mass and lipid mass resulted when voles were provided warm (23° C) conditions with long day (16L:8D) photoperiod and fed high-fat food (Figure 5.10, 5.11). In contrast, the smallest gains in total body mass and lipid mass occurred under cold (5° C) conditions with short day (8L:16D) photoperiod and fed lab chow (Figure 5.10, 5.11). When comparing voles held under identical conditions (5° C , 8L:16D) but fed different diets, voles eating the high-fat diet had significantly more ($p < 0.01$) lipid mass (16.24 ± 1.64 g) compared to voles eating lab chow (9.39 ± 3.07 g) (Figure 5.11). Thus, the high-fat diet results in larger gains in body fat. Comparing voles eating the same diet (high-fat) under different environmental conditions, the long day, warm condition voles showed significantly more lipid mass (22.82 ± 1.49 g) by week 10 than voles on short days in the cold ($p < .02$) (Figure 5.11). This response suggests less environmental stress due to temperature and photoperiod under long day, warm conditions. In all groups, changes in fat-free mass over the 10 week period were minimal, with no significant difference

between treatments at any time (Figure 5.12). These results suggest no effect due to gutfill. This was expected since no there was no reduction in dietary quality and unlimited food was available. In addition, voles did not decrease body mass in response to changing photoperiod.

DISCUSSION

With this series of experiments, we showed that significant change in body composition results when meadow voles are removed from the field, held under stable laboratory conditions and fed normal lab chow. Regardless of season, the pattern of body composition and change is consistent. In all experiments, voles increased body mass and that increase was due to large gains in lipid mass, combined with small losses in fat-free mass. Changes in body fat in our study are similar to those reported by Ferns and Adams (1974), Sawicka-Kapusta (1974) and Batzli and Esseks (1992) for microtines removed from the field, but none of these authors reported changes in fat-free mass. Ferns and Adams (1974) reported that after three weeks in the lab, *M. agrestis* increased body fat content 4-5 times the body fat content of voles captured in the field. Sawicka-Kapusta (1970, 1974) showed that body fat content of laboratory *M. arvalis* was > 10 % after 20 days of age and that wild caught *Clethrionomys glareolus* reached levels > 10 % body fat by 30 days of age when kept in laboratory cages. In a study evaluating the response of brown lemmings (*Lemmus sibiricus*) removed from the field and held in the laboratory, Batzli and Esseks (1992) found that animals increased body fat from field levels of 3 to

5 % up to 9 to 13 % in the lab when fed natural vegetation. When fed unnatural, highly digestible food (rabbit chow), brown lemmings further increased body fat to over 30 %. In our study, we were unable to confirm body composition change in meadow voles when fed natural vegetation due to high mortality in a short time period. Hollemann and Dieterich (1978) also reported that body fat exceeded 10 % after three weeks of age, with values as high as 44 % in a laboratory colony of brown lemmings eating lab chow.

Batzli and Esseks (1992) also reported that food intake was positively correlated with body fat. In contrast, we found that food intake actually decreased over time as voles became fatter. Since fat is less metabolically active than fat-free mass, increased body fat should not require greater increases in food intake once fat is deposited. In addition, since voles were simultaneously losing fat-free mass, metabolic demands may have been decreasing even though voles were getting bigger. However, as previously mentioned, some of the decrease in fat-free mass levels may be due to gut content. As a result, a reduction in food intake would result, as we observed.

When voles were fed lab chow (5 % fat) or rabbit chow (1.5 % fat), they increased body fat from less than 5 % to 25 % within six weeks. By further increasing dietary fat to 25% (high-fat diet), voles gained additional body fat to levels exceeding 30 %. However, regardless of diet, voles did not continue to increase body mass. Rather, they showed a plateau in body mass for each diet. In all cases, the majority of lipid deposition occurred within two to three weeks in the lab, followed by a leveling in all body composition parameters for the remaining time. When fat content of the diet was changed, either increased or decreased, voles responded by increasing or decreasing body

composition parameters. In experiment 3, when dietary fat was increased with high-fat food, voles gained additional body and lipid mass, and when dietary fat was decreased with lab chow from the high-fat diet, voles lost body and lipid mass. We interpret these changes in body composition as responses to diet, where voles regulate body mass and body composition at levels which correspond to dietary quality and abundance. Unlike laboratory rats, which continue to deposit added body fat without limit when given *ad lib.* food (Donald et al. 1980), the voles in our study reached certain body composition plateaus associated with diet quality. Since we did not observe continual diet induced obesity, our results suggest some regulatory mechanism in overall body composition of voles. In all cases, gains in total body mass were less than net increases in lipid mass. Thus, small losses in fat-free mass also occurred contributing to the overall change in body mass. Our data suggest that voles are regulating body mass by differentially varying lipid and fat-free mass in relation to diet.

There are many possible explanations for higher body fat levels observed when animals are held in the laboratory. Paramount among these are: an unnatural highly digestible food; a reduction in activity lessening overall energy demands; or stable environmental conditions reducing energy demands for thermoregulation; or some interaction of these. Results from our experiments indicate that food quality has a strong effect on resulting body composition in the laboratory. In experiment 5, voles which consumed high-fat food had higher levels of body fat, regardless of temperature or photoperiod condition. Voles held under long day, warm conditions (16L:8D, 23° C) and fed a high-fat diet showed the greatest gains in body mass and body fat (Figure 5.9, 5.10).

When maintained under short day, cold conditions (8L:16D, 5° C), voles eating the high-fat diet showed greater increases in body mass and body fat than the voles eating lab chow (Figure 5.9, 5.10). In contrast to laboratory studies where microtines decrease body mass in response to decreasing temperature and shortened photoperiod (Dark and Zucker 1983; Dark et al. 1989; Peterborg 1978; Pinter 1968; Pistole and Cranford 1982; Rhodes 1989; Vaughan et al. 1973) voles in our study were still able to increase body mass and body fat when exposed to simulated winter conditions (8L:16D, 5° C) (Figure 5.9, 5.10). Pistole and Cranford (1982) showed that adult voles lost mass continuously (0.05 g/day) under decreasing photoperiod and temperature until winter solstice. Sealander (1966) reported a high correlation between ambient temperature, photoperiod and body weight for *Clethrionomys rutilus* in the field. Meadow voles lost 20 % of body mass with reduced photoperiod, regardless of temperature (23° C vs. 10° C) (Dark and Zucker 1983). In addition, meadow voles in their study decreased energy intake by 30 % when photoperiod was reduced. Our results suggest that abundant high quality food may reduce the effects of decreasing temperature and photoperiod on physiologic changes in body composition. Although we did not directly measure food intake within experiment 5, the increased thermogenic demands associated with lower temperatures may have been met by unconstrained intake of high quality food. We also feel that there may be a differential body composition response to changing environmental conditions based on zoogeography. Changes in body composition in small mammals, and in particular microtines, may vary by locale. The scale of changes in body composition which result in response to changing environmental conditions may vary differentially dependent upon the severity of the

environmental change. Differing changes in body composition in response to photoperiod and temperature change within the same species suggests a possible latitudinal effect where small mammals vary body composition dramatically in arctic regions (Peterborg 1978; Pinter 1980; Vaughan et al. 1973), with a lesser response when found in more temperate environments (Berbehenn 1955; Brown 1973; Iverson and Turner 1974). As a result, we feel that meadow voles collected in southeastern Colorado may respond differently than those collected in more northern regions of the United States (Dark and Zucker 1983; Pistole and Pistole and Cranford 1982) and Canada.

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Table 5.1. Fat content of wild, small mammals. Reported as percent body fat.

Species	Fat Content	Reference
Meadow vole (*Microtus pennsylvanicus*)	3 - 7	1
	3 - 8	11
Pine vole (*Microtus pinetorum*)	5 - 7	7
Prairie vole (*Microtus ochrogaster*)	4 - 5	8
	3 - 5	10
	4 - 6	2
Red-backed voles (*Clethrionomys gapperi*)	3 - 5	1
Brown lemming (*Lemmus sibiricus*)	3 - 5	3
Deer mouse (*Peromyscus maniculatus*)	3 - 5	9
	5 - 7	5, 8
White-footed mouse (*Peromyscus leucopus*)	5 - 8	6
Harvest Mouse (*Reithrodontomys megalotis*)	3 - 5	9
	6 - 7	5
Grasshopper mouse (*Onychomys leucogaster*)	4 - 6	9
Cotton rat (*Sigmodon hispidus*)	5 - 10	5
Plains pocket gopher (*Geomys bursarius*)	4 - 8	4
Yellow-faced pocket gopher (*Pappageomys castanops*)	4 - 9	4

1. Anderson and Rauch 1984
2. Baker 1971
3. Batzli and Esseks 1992
4. Fehrenbacher and Fleharty 1976
5. Fleharty et al. 1973
6. Lynch 1973
7. Lochmiller et al. 1983
8. Morton and Lewis 1980
9. Schreiber and Johnson 1975
10. Voltura 1997
11. This study

Table 5.2. Detailed composition of lab chow and rabbit chow. Data provided by PMI Feeds and is based on the latest ingredient analysis information. Since nutrient composition of ingredients varies, analyses will vary accordingly.

	Lab Chow PMI 5001	Rabbit Chow PMI 5325
Guaranteed Analysis		
Protein, minimum %	23.0	14.0
Fat, minimum %	4.5	1.5
Fiber, maximum %	6.0	25.0
Ash, maximum %	NR	10.0
Added minerals, maximum %	NR	1.5
Chemical Composition		
Protein %	23.40	14.50
Fat %	4.50	1.70
Fiber (Crude) %	5.80	22.50
Neutral Detergent Fiber %	16.00	40.90
Acid Detergent Fiber %	8.20	24.60
Total Digestible Nutrients %	76.00	57.00
Gross energy, (Kcal/g)	4.25	3.90
Physiologic Fuel Value (Kcal/g)	3.30	2.43
Ash %	7.30	8.90
Phosphorus %	0.61	0.50
Potassium %	1.10	1.67
Sodium %	0.40	0.32

1) Nutrient expressed as percent of ration except as noted. Moisture content is assumed to be 10% for the purpose of calculations
2) NDF = approximately cellulose, hemicellulose, and lignin
3) ADF = approximately cellulose and lignin
4) Physiological Fuel Value = sum of decimal fractions of protein x 4, fat x 9, and carbohydrate x 4 Kcal/gm respectively

Table 5.3. Summary of the proximate analysis of the high-fat diet (lab chow + fat) fed to voles in the September group. Column values represent composition as received (A). Cake were further dried to constant mass by the analysis lab and reported on a dry matter basis (B). Lab chow data reported on a dry matter basis by PMI Feeds and is uncertified. NR= not reported by testing source.

	Lab Chow + Fat		Lab Chow
	A	B	B
%			
Moisture	14.75	0.00	10.00
Dry Matter	85.25	100.00	100.00
Crude Protein	16.75	19.70	23.4
ADF	5.24	6.16	8.2
Nitrogen	2.68	3.15	NR
Phosphorus	0.45	0.53	0.61
Potassium	0.92	1.08	1.10
Calcium	0.71	0.84	1.00
Magnesium	0.14	0.17	0.21
Sodium	3.06	3.60	0.40
mg/kg (ppm)			
Iron	189.3	222.7	198.0
Manganese	46.15	54.29	64.3
Zinc	54.91	64.60	70.0
Copper	10.43	12.27	18.0
Boron	7.36	8.66	NR
Molybdenum	0.95	1.12	NR

Table 5.4. Summary of the proximate analysis of vegetation collected in October and fed to voles. One 500 g wet sample was analyzed. Column values represent composition as received (A). Sample was further dried to constant mass by the analysis lab and reported on a dry matter basis (B). Lab chow data reported on a dry matter basis by PMI Feeds and are uncertified. NR= not reported by testing source.

	Vegetation		Lab Chow
	A	B	
%			
Moisture	44.38	0.00	10.00
Dry Matter	55.62	100.00	100.0
Crude Protein	4.66	8.32	23.4
ADF	26.03	46.49	8.2
Nitrogen	0.74	1.33	NR
Phosphorus	0.05	0.09	0.61
Potassium	0.52	0.93	1.10
Calcium	0.28	0.50	1.00
Magnesium	0.06	0.11	0.21
Sodium	0.04	0.08	0.40
mg/kg (ppm)			
Iron	66.08	118.0	198.0
Manganese	105.8	188.9	64.3
Zinc	18.06	32.25	70.0
Copper	4.27	7.63	18.0
Boron	1.62	2.89	NR
Molybdenum	2.77	4.94	NR

Table 5.5. Comparison of body composition parameters for seasonal groups fed lab chow (Lab Chow 5001, PMI Feeds) and water ad lib. Values are mean plus/minus one standard error (in parentheses). ANOVA F value indicates degree of similarity or difference for three groups for each body composition parameter over two week periods. No significant differences were found for any body composition parameter between groups in any time period.

Time & Condition	F Value ANOVA	January 97 n=12	April 97 n=9	June 97 n=10
Start to Week 2				
Mass change (g)	.9736	4.30 (1.31)	4.70 (2.38)	4.16 (1.24)
Lipid mass change (g)	.4617	5.90 (0.58)	6.22 (1.48)	7.40 (0.60)
Fat-free mass change (g)	.5743	-1.85 (1.13)	-1.52 (1.39)	-3.20 (0.94)
Start to Week 4				
Mass change (g)	.9806	6.40 (1.96)	5.84 (2.17)	6.28 (2.09)
Lipid mass change (g)	.6936	8.41 (1.19)	8.81 (1.46)	9.86 (1.10)
Fat-free mass change (g)	.5277	-2.27 (1.09)	-4.22 (1.07)	-3.54 (1.51)
Start to Week 6				
Mass change (g)	.7245	6.18 (2.18)	8.90 (1.79)	7.08 (2.97)
Lipid mass change (g)	.6841	8.84 (1.21)	10.29 (1.12)	10.40 (1.85)
Fat-free mass change (g)	.7400	-2.95 (1.30)	-2.50 (1.17)	-4.29 (2.27)

Table 5.6; Summary of body composition parameters for groups fed different diets: lab chow (Lab Diet 5001, PMI Feeds) and rabbit chow (Lab Diet 5325, PMI Feeds). All values are mean plus/minus one standard error (in parentheses). There were no significant differences between groups for any parameter in any time period.

Time & Condition	Lab Chow (5001) n=9	Rabbit Chow (5325) n=5
Start to Week 2		
Mass change (g)	4.70 (2.38)	2.78 (1.41)
Lipid mass change (g)	6.22 (1.48)	5.38 (1.02)
Fat-free mass change (g)	-1.52 (1.39)	-4.06 (2.61)
Start to Week 4		
Mass change (g)	5.84 (2.17)	4.59 (1.30)
Lipid mass change (g)	8.81 (1.46)	6.42 (1.22)
Fat-free mass change (g)	-4.22 (1.07)	-1.83 (2.12)
Start to Week 6		
Mass change (g)	8.90 (1.79)	6.16 (1.13)
Lipid mass change (g)	10.29 (1.12)	8.26 (2.11)
Fat-free mass change (g)	-2.50 (1.17)	-2.10 (2.57)

Table 5.7. The effects of food quality on body composition. Group A was fed lab chow (Lab Diet 5001, PMI Feeds) for six weeks with group B fed high-fat diet. After six weeks, diet was switched for an additional three weeks. All values are mean plus/minus one standard error (in parentheses). * indicates significant difference from body composition parameter at start of experiment. ** indicates significant difference from body composition parameter at week 6 following change in diet ($p < .05$).

Time & Condition	Group A Lab Chow	Group B High-Fat
Start to Week 6		
Mass change (g)	7.08 (2.97)*	5.74 (2.08)*
Lipid mass change (g)	10.40 (1.85)*	13.18 (0.94)*
Fat-free mass change (g)	-4.29 (2.27)*	-7.44 (1.53)*
	Diet Change	
	High-Fat	Lab Chow
Week 6 to Week 9 (Diet Change)		
Mass change (g)	5.86 (0.81)**	-8.69 (1.22)**
Lipid mass change (g)	2.99 (0.55)**	-6.41 (1.81)**
Fat-free mass change (g)	2.88 (0.57)**	-3.52 (1.02)**

Table 5.8. Summary of food intake rates for groups fed different diets. Values are mean plus/minus one standard error (in parentheses). * indicates significant differences between consecutive weeks within a group ($p < .05$)

Week	Group A Lab Chow	Group B High-Fat
Week 1		
Intake/d (g)	8.04 (0.48)	6.30 (0.64)
Intake/g body mass (g/g^{-1})	0.18 (0.01)	0.12 (0.01)
Week 2		
Intake/d (g)	8.57 (0.37)*	6.30 (0.64)
Intake/g body mass (g/g^{-1})	0.18 (0.01)	0.12 (0.01)
Week 3		
Intake/d (g)	7.02 (0.38)*	5.77 (0.38)
Intake/g body mass (g/g^{-1})	0.14 (0.01)	0.10 (0.00)
Week 4		
Intake/d (g)	6.48 (0.30)	4.82 (0.23)
Intake/g body mass (g/g^{-1})	0.13 (0.01)	0.09 (0.00)
Week 5		
Intake/d (g)	6.67 (0.34)	4.96 (0.27)
Intake/g body mass (g/g^{-1})	0.13 (0.01)	0.09 (0.00)
Week 6		
Intake/d (g)	6.13 (0.30)	5.22 (0.35)
Intake/g body mass (g/g^{-1})	0.12 (0.01)	0.09 (0.01)
	Diet Change	
Week 7		
Intake/d (g)	5.55 (0.31)	5.63 (0.38)
Intake/g body mass (g/g^{-1})	0.10 (0.01)	0.11 (0.01)
Week 8		
Intake/d (g)	4.50 (0.21)	5.71 (0.23)
Intake/g body mass (g/g^{-1})	0.08 (0.00)	0.12 (0.00)
Week 9		
Intake/d (g)	4.54 (0.14)	5.71 (0.23)
Intake/g body mass (g/g^{-1})	0.08 (0.00)	0.12 (0.00)

Figure 5.1. Body composition change of voles captured in January and fed lab chow (Lab Diet 5001, PMI Feeds) and water *ad lib* . for six weeks. Values represented are mean plus/minus one standard error (error bars). Different numbers indicate a significant difference between consecutive weeks ($p < .05$). * indicates a significant difference by week from the parameter value at the start of the experiment ($p < .05$).

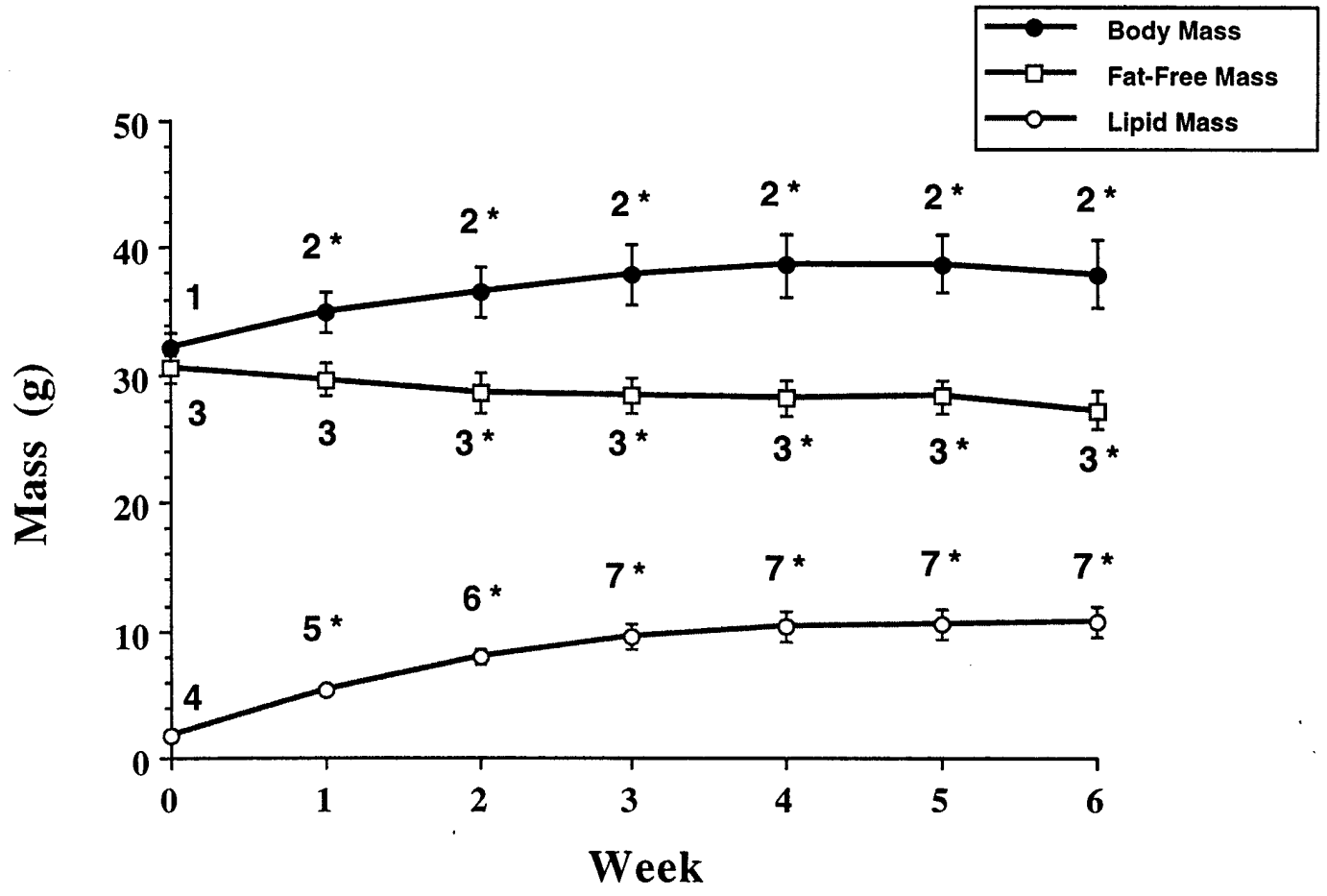


Figure 5.2. Body composition change of voles captured in April and fed lab chow (Lab Diet 5001, PMI Feeds) and water *ad lib* . for six weeks. Values represented are mean plus/minus one standard error (error bars). Different numbers indicate a significant difference between consecutive weeks ($p < .05$). * indicates a significant difference by week from the parameter value at the start of the experiment ($p < .05$).

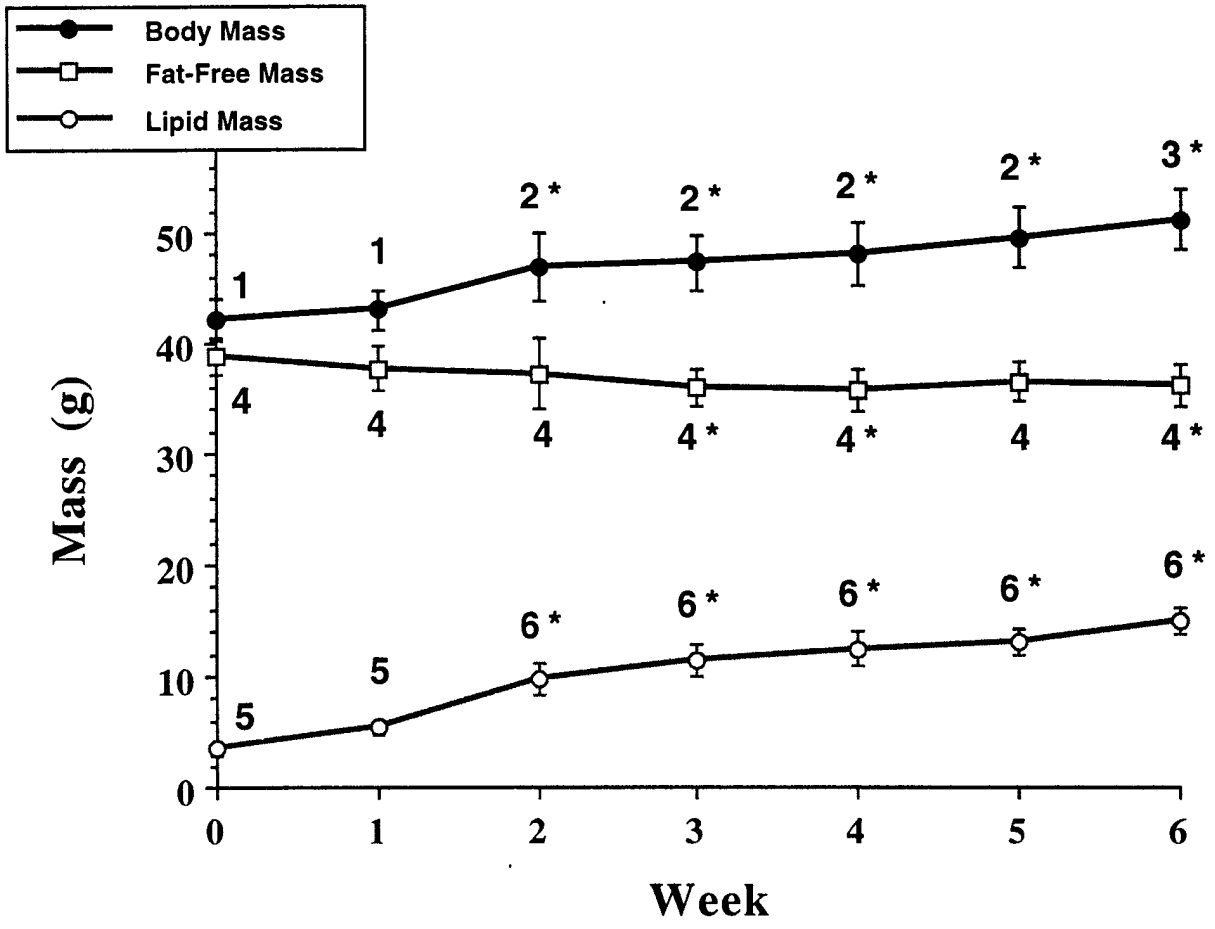


Figure 5.3. Body composition change of voles captured in June and fed lab chow (Lab Diet 5001, PMI Feeds) and water *ad lib.* for six weeks. Values represented are mean plus/minus one standard error (error bars). Different numbers indicate a significant difference between consecutive weeks ($p < .05$). * indicates a significant difference by week from the parameter value at the start of the experiment ($p < .05$).

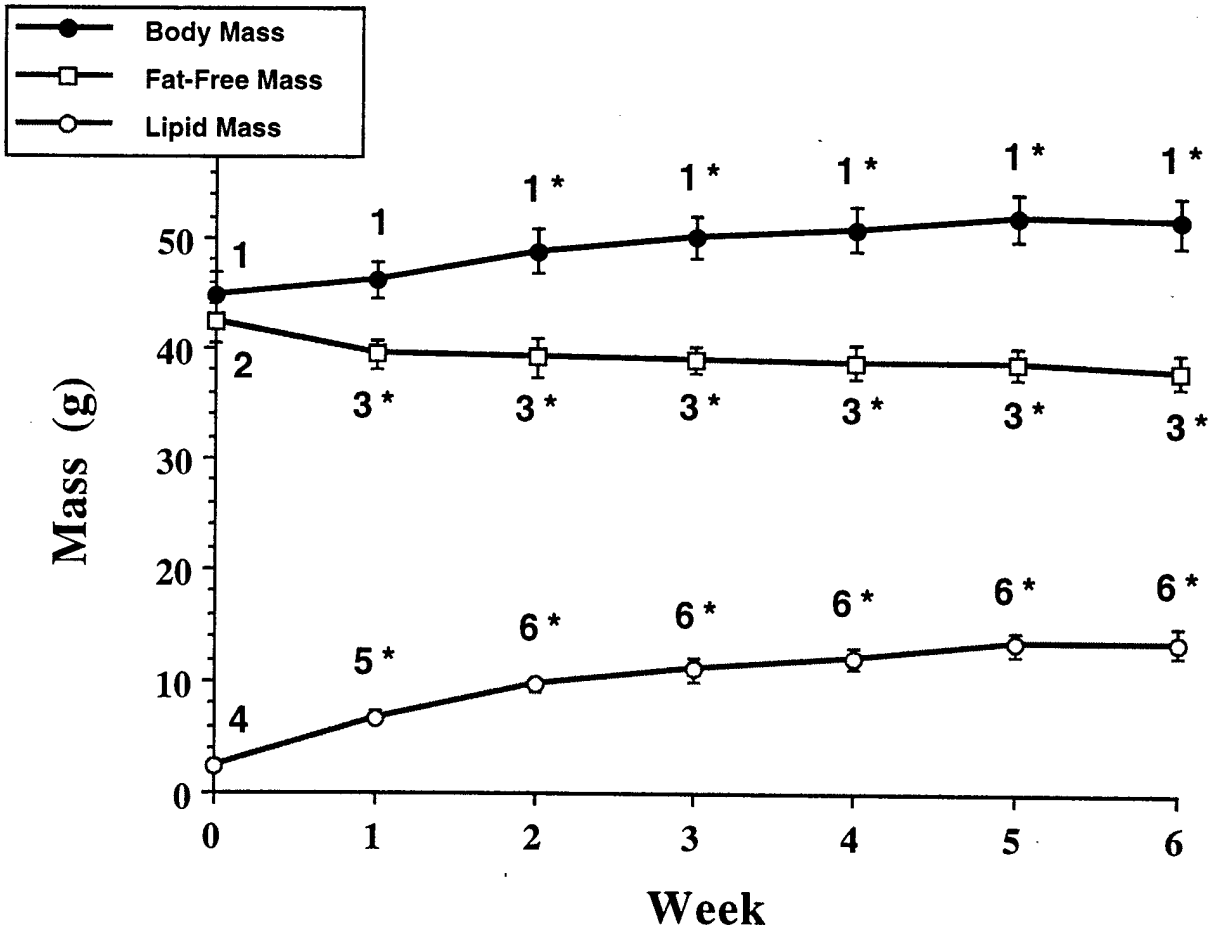


Figure 5.4. Comparison of percent lipid mass for all groups of voles fed lab chow (Lab Diet 5001, PMI Feeds) and water *ad lib* . for six weeks. Values represent mean plus/minus one standard error (error bars). Different letters indicate significant differences between groups at each week ($p < .05$). Different numbers indicate significant differences between consecutive weeks ($p < .05$).

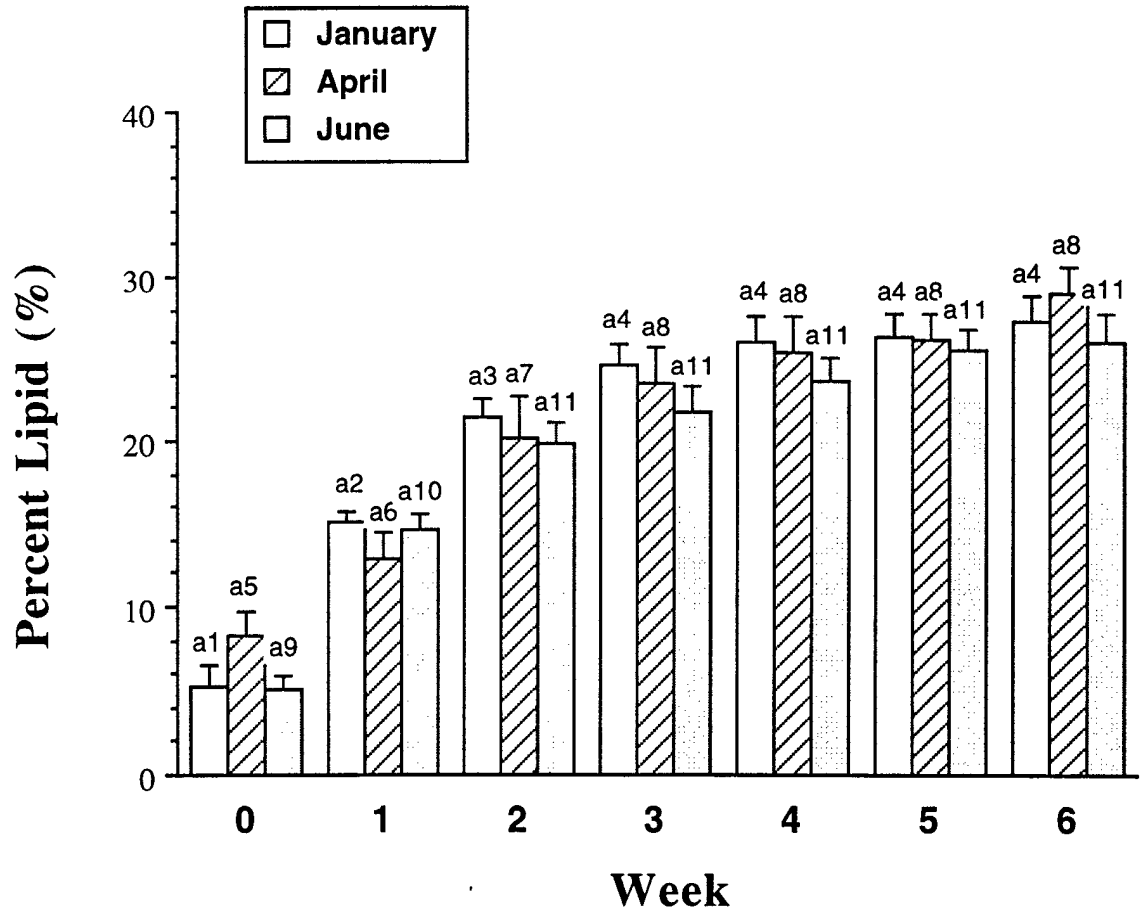


Figure 5.5. Comparison of percent lipid mass for groups of voles fed either lab chow or rabbit chow for six weeks. Values represented are mean plus/minus one standard error (error bars). Different letters indicate significant differences between consecutive weeks within a treatment ($p < .05$). There were no significant differences in percent lipid between treatments during any week ($p > .05$).

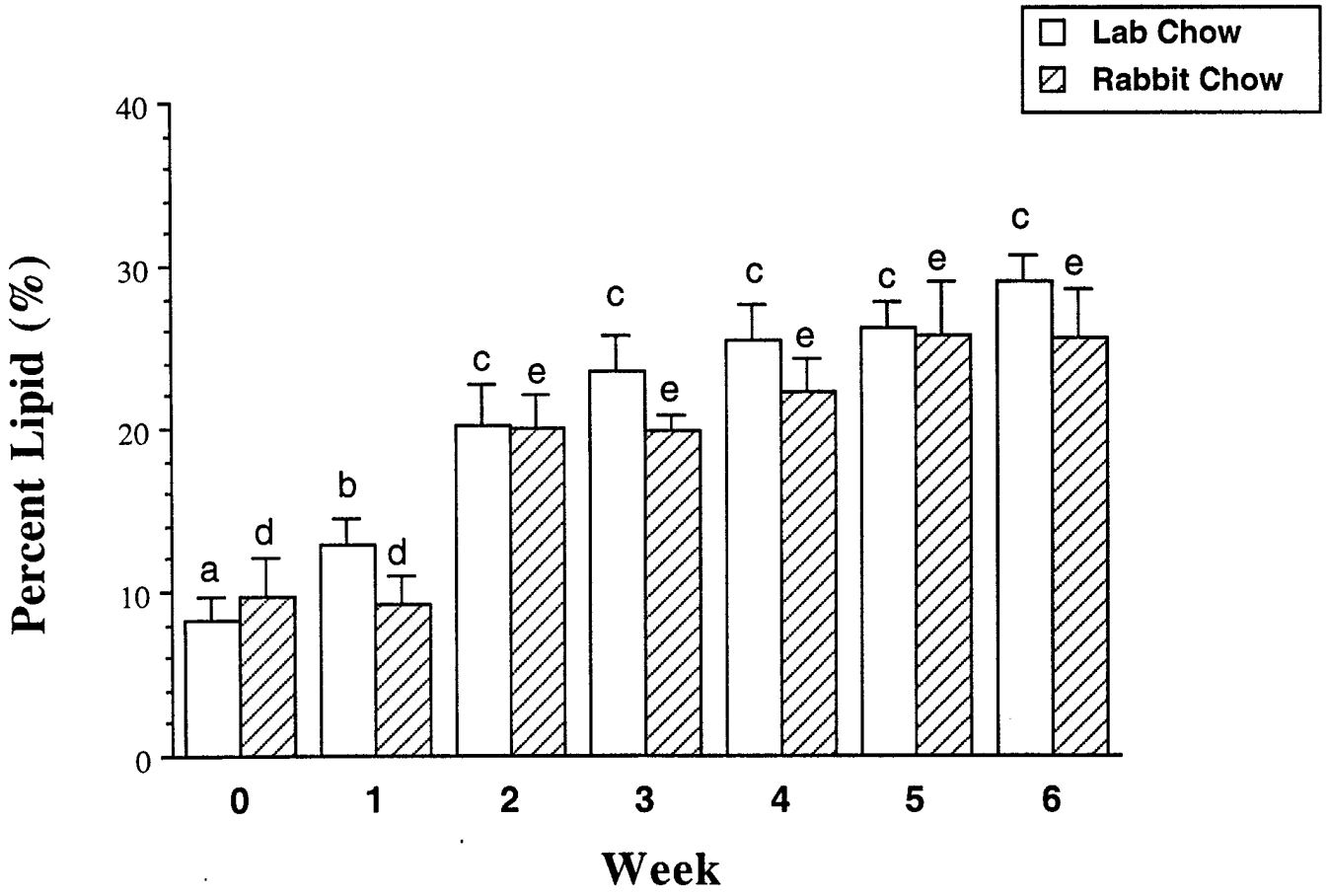


Figure 5.6. Comparison of daily food intake for voles fed lab chow or rabbit chow. Values represented are mean plus/minus one standard error (error bars). Significant differences ($p < .05$) between groups designated by *. Different letters indicate significant differences between consecutive weeks within each treatment ($p < .05$).

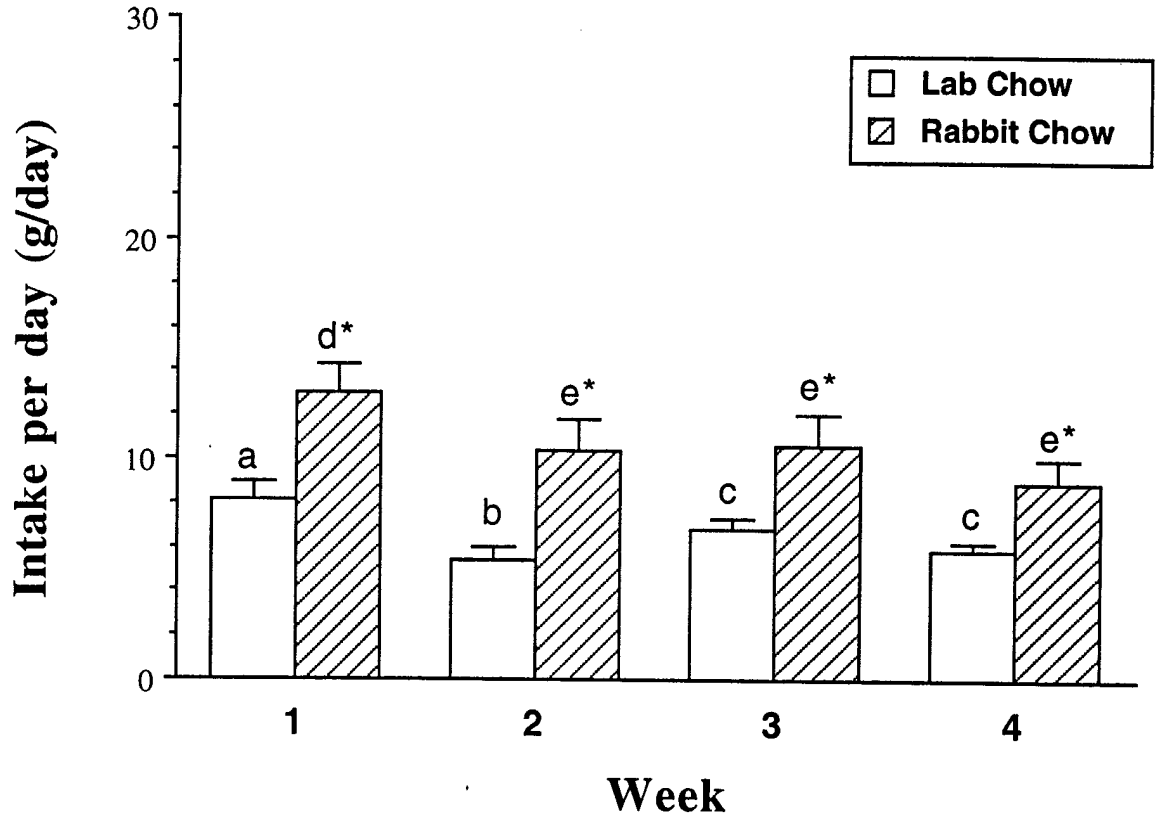


Figure 5.7. Change in body mass (A) and change in lipid mass (B) in comparison to food intke (g/day) for voles fed lab chow and rabbit chow.

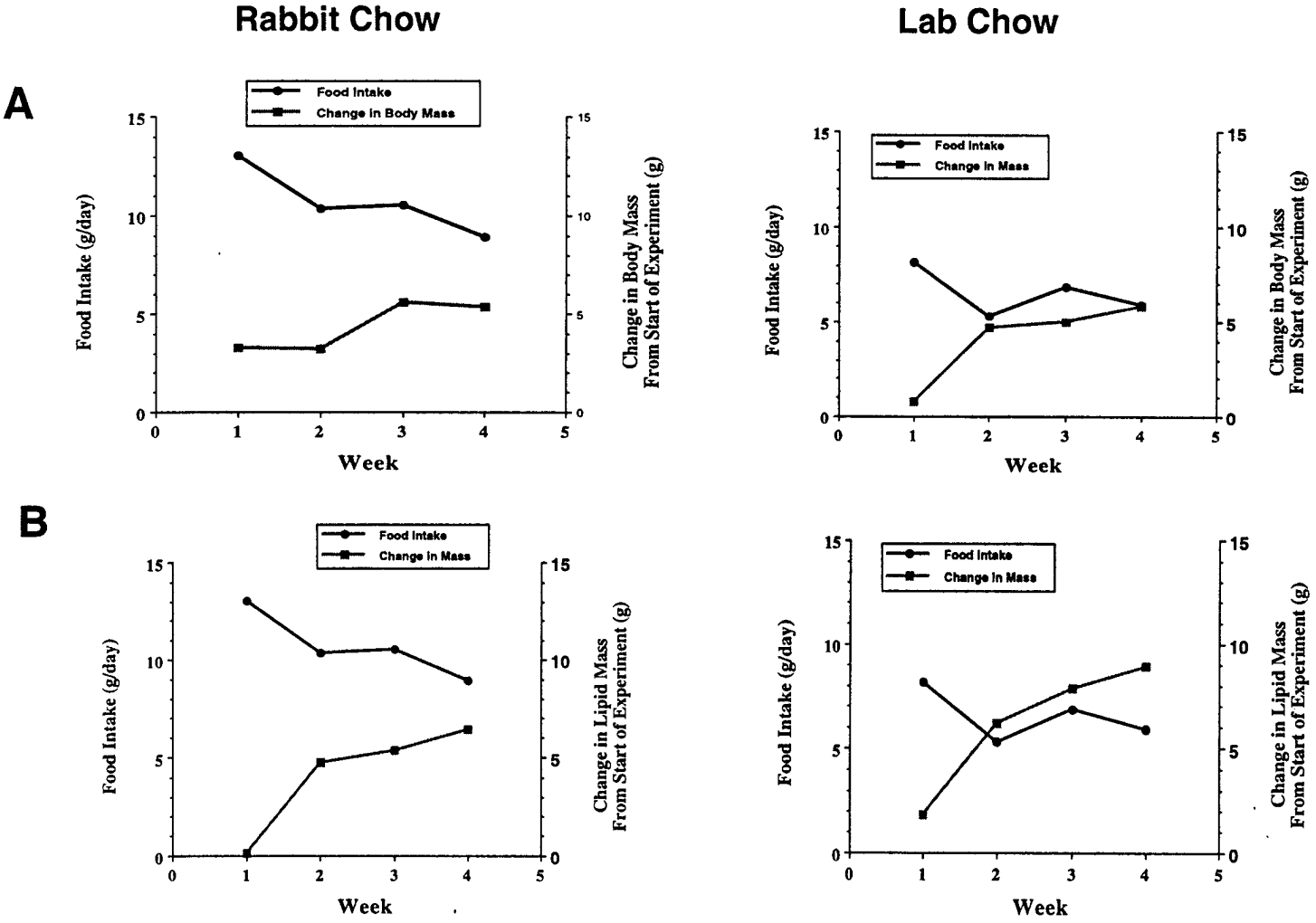


Figure 5.8. Body composition change for the June voles fed lab chow for six weeks, then a high-fat diet for an additional three weeks. Values represent mean plus/minus one standard error (error bars). Different numbers indicate a significant difference between consecutive weeks ($p < .05$) * indicates a significant difference from parameter value at start of experiment ($p < .05$). a indicates a significant difference from parameter value at week 6 ($p < .05$).

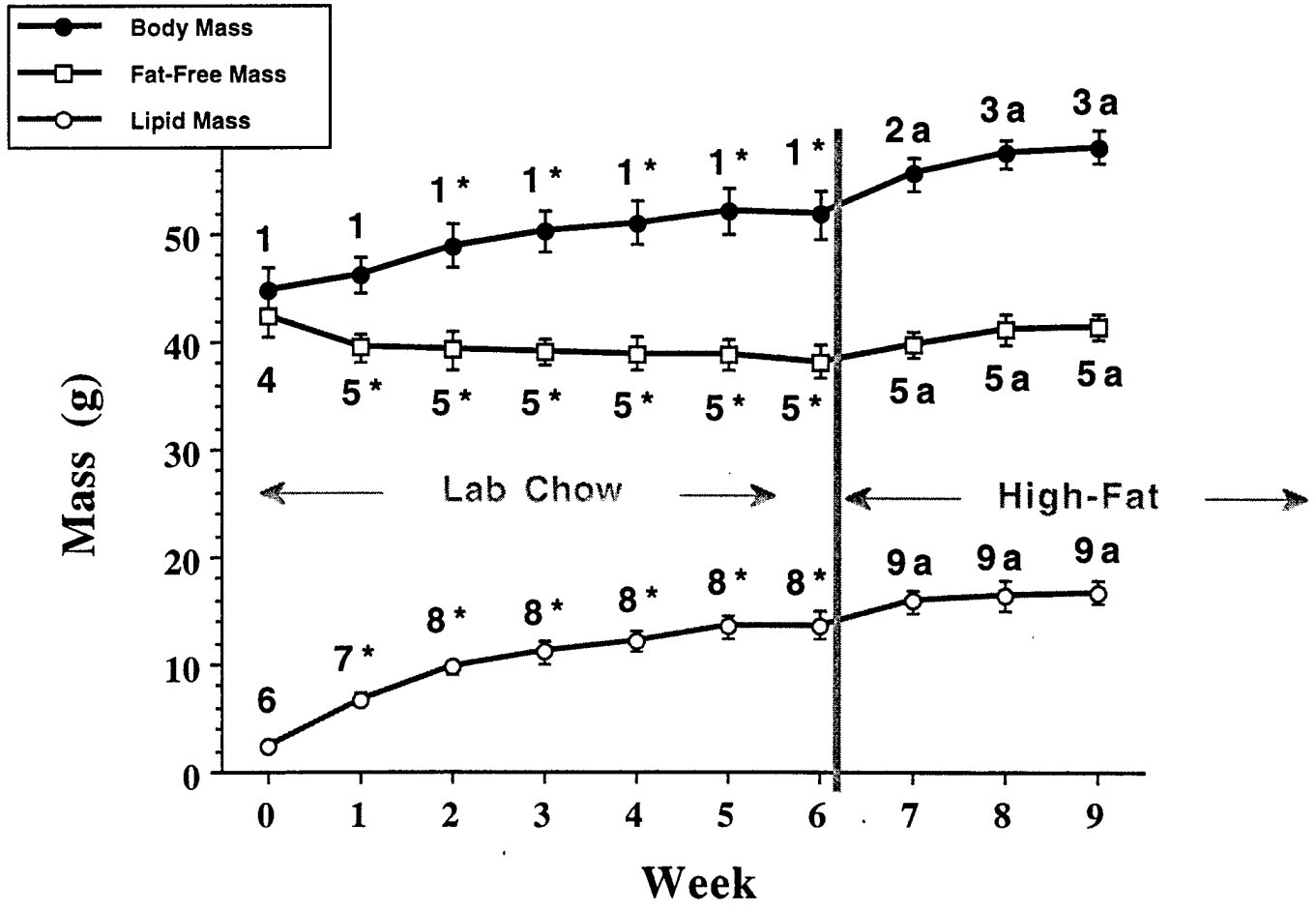


Figure 5.9. Body composition change for the September voles fed a high-fat diet for six weeks, then lab chow for an additional three weeks. Values represent mean plus/minus one standard error (error bars). Different numbers indicate a significant difference between consecutive weeks ($p < .05$) * indicates a significant difference from parameter value at start of experiment ($p < .05$). a indicates a significant difference from parameter value at week 6 ($p < .05$).

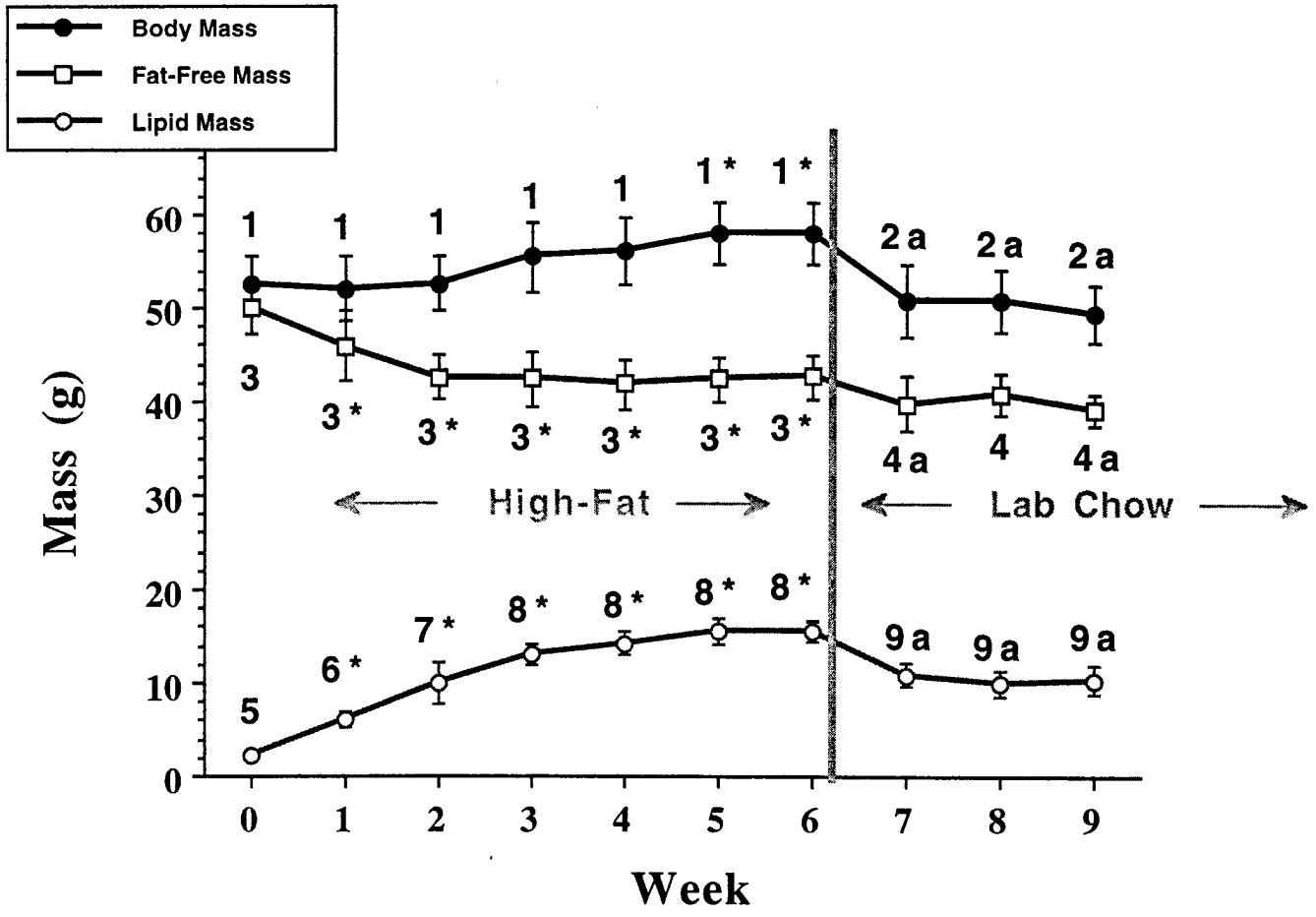
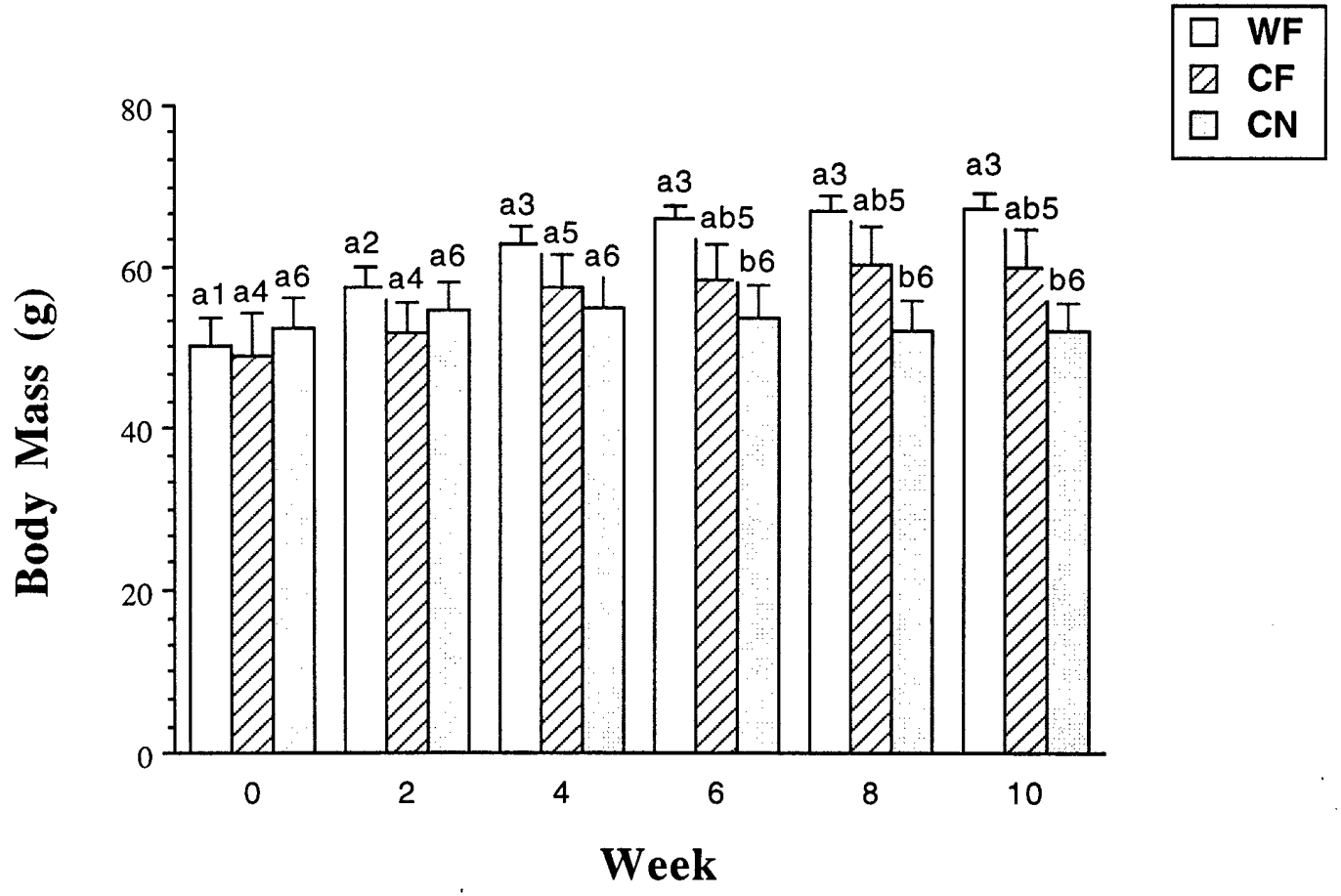
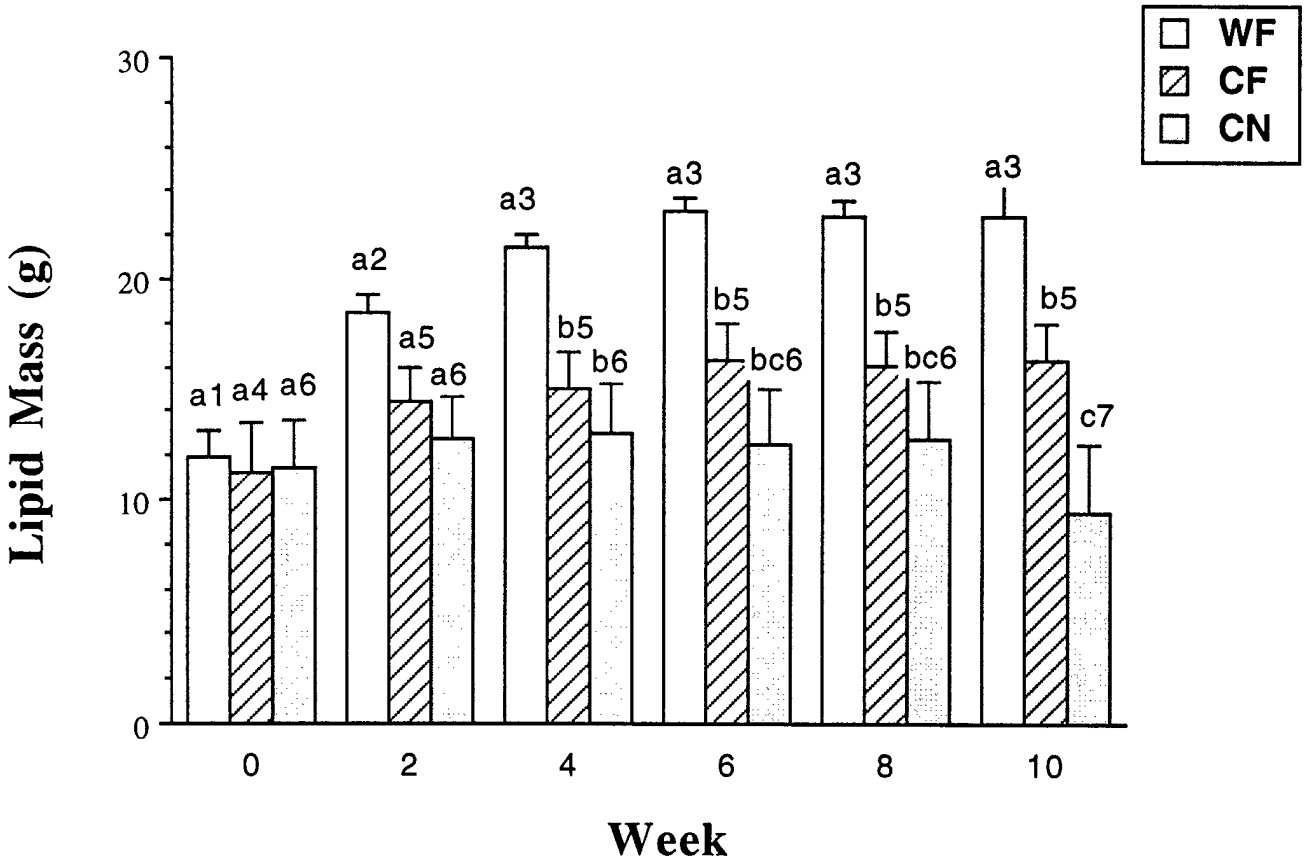


Figure 5.10. Comparison of total body mass for all treatments over a 10 week period. Values are mean plus/minus one standard error (error bars). Different letters indicate significant differences between treatments at each week interval. Different numbers indicate significant differences between consecutive weeks within each treatment ($p < .05$).



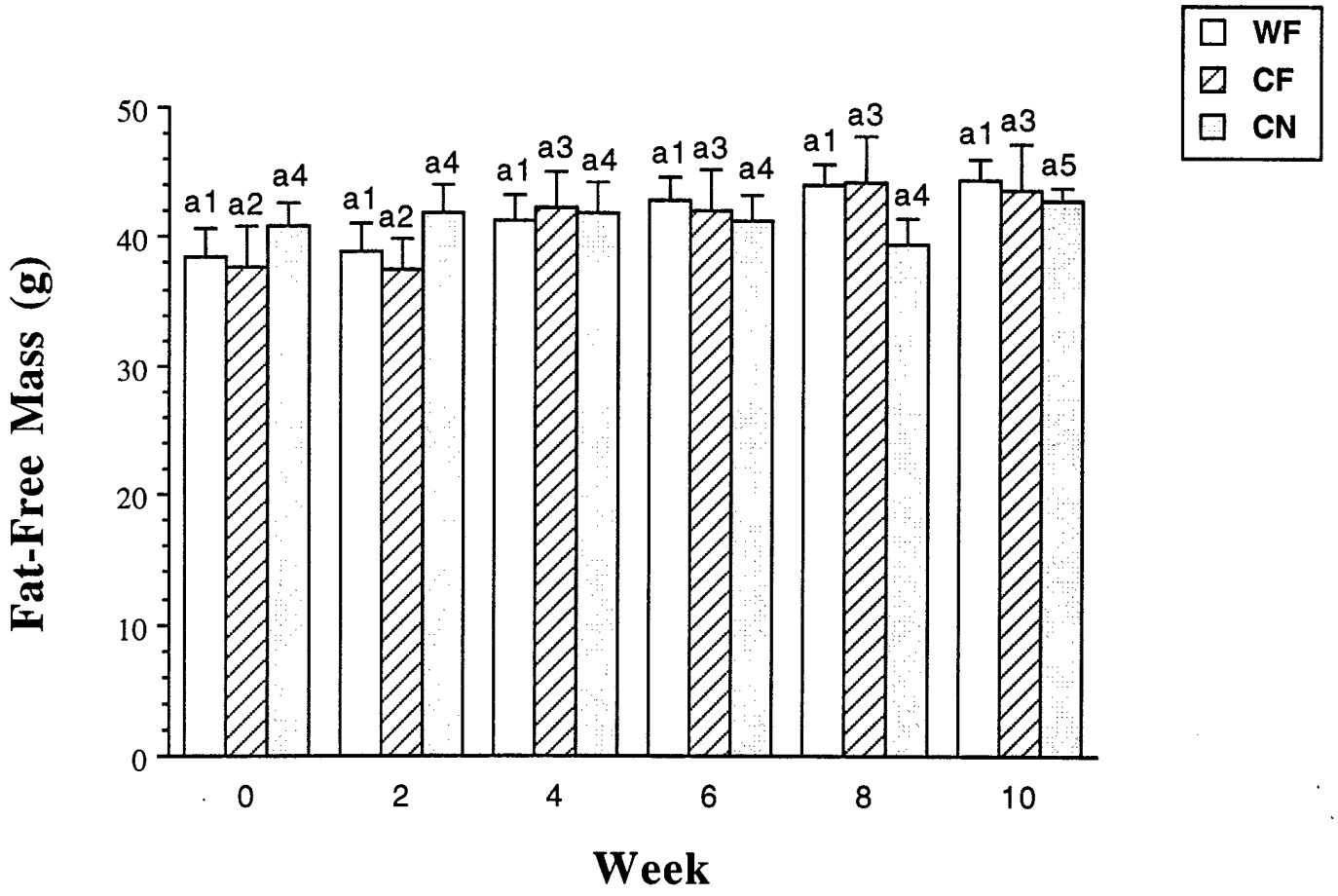
CN = 8L:16D, 5°C; lab chow diet (Group A)
CF = 8L:16D, 5°C; high-fat diet (Group B)
WF = 16L:8D, 23°C; high-fat diet (Group C)

Figure 5.11. Comparison of lipid mass for all treatments over a 10 week period. Values are mean plus/minus one standard error (error bars). Different letters indicate significant differences between treatments at each week interval. Different numbers indicate significant differences between consecutive weeks within each treatment ($p < .05$).



CN = 8L:16D, 5°C; lab chow diet (Group A)
CF = 8L:16D, 5°C; high-fat diet (Group B)
WF = 16L:8D, 23°C; high-fat diet (Group C)

Figure 5.12. Comparison of fat-free mass for all treatments over a 10 week period. Values are mean plus/minus one standard error (error bars) Different letters indicate significant differences between treatments at each week interval. Different numbers indicate significant differences between consecutive weeks within each treatment ($p < .05$).



CN = 8L:16D, 5°C; lab chow diet (Group A)
CF = 8L:16D, 5°C; high-fat diet (Group B)
WF = 16L:8D, 23°C; high-fat diet (Group C)

Chapter 6

Preference for dietary fat in the meadow vole, *Microtus pennsylvanicus*

INTRODUCTION

The meadow vole (*Microtus pennsylvanicus*) is a vegetation herbivore that lives in a variety of open habitats (Batzli 1985). They are known to be selective feeders (Batzli and Pitelka 1971; Bergeron and Jodoin 1987; Zimmerman 1965), but the bulk of their diet in nearly entirely monocots (Batzli and Pitelka 1971) or dicots of varying digestibility (Caron et al. 1985). There is evidence that voles face multiple constraints from the foods they ingest: low or high contents of proteins and fibers (Goldberg et al. 1980; Harju and Hakkarainen 1997; Keys and VanSoest 1970), secondary metabolites (Bergeron and Yean 1986; Bergeron et al. 1987; Lindroth and Batzli 1984), alkaloids (Kendall and Sherwood 1975; Kendall and Leath 1976; Lindroth and Batzli 1986), as well as minerals (Schultz 1969; Freeland et al. 1985; Christian 1989). High crude protein and low phenolic contents of diets (Lindroth and Batzli 1984; Lindroth et al. 1984), as well as high levels of nonstructural carbohydrates (Servello et al. 1983) are believed to be good indicators of diet quality.

Numerous studies have shown associations between specific plant constituents and food habits, reproduction, or growth patterns of meadow voles. However, no one has investigated the effects of dietary fat on food selection. In the wild, body fat in many small, non-hibernating rodents is low, often ranging from 3 to 8 % of total body mass (Batzli and Esseks 1992; Didow and Hayward 1969; Fleharty et al. 1973; Lynch 1973;

Morton and Lewis 1980; Nestler et al. 1996; Rock and Williams 1979; Sawicka-Kapista 1970; Schreiber and Johnson 1975; Voltura 1997) (Table 6.1). However, when brought into the lab, they get fat (Batzli and Esseks 1992; Ferns and Adams 1974; Voltura and Wunder 1998). Thus, this lean body composition may be the result of a diet which is low in available fat. Monocots usually contain less than 2 % available fat (NRC 1964). As a consequence, meadow voles usually eat a low fat diet. However, since they do fatten in the lab, we tested whether they could select a high-fat diet when given a choice.

MATERIALS AND METHODS

Food preference

Foods offered were lab chow (Lab Diet 5001, PMI Feeds) or lab chow plus added vegetable oil to increase the overall fat content (high-fat). Lab chow contains approximately 5 % total fat (reported by PMI Feeds), while the high-fat diet mixture was calculated to be approximately 25 % fat (Chapter 5). Food was offered as patties or cakes. We used ground lab chow mixed with water (4:1 by volume) to form a paste and then pressed into cakes with a quarter pound hamburger press. For the high-fat diet, lab chow was mixed with both water (4:1 by volume) and vegetable oil (4:2 by volume) and formed into cakes using identical procedures. To minimize fat volatilization, both diets were then baked at 100° C for four hours and dried for an additional 24 hours at 50° C. Our goal was to remove as much water as possible and to make both diets as similar as possible in texture and appearance, with variation only in fat quantity. Since we expected slight mineral and protein dilution in the high-fat diet, a proximate analysis (Nahm 1992)

was performed by the Soil, Water, and Forage Analysis Lab at Colorado State to document the degree of diet dilution. Composition comparisons between diets are presented in Table 6.2.

Random food position test

Ten meadow voles, approximately 60 days old, were obtained from our breeding colony at Colorado State University maintained at 23° C and a 16L:8D photoperiod. Voles were maintained in large individual cages (43 x 21 x 20 cm) on a 16L:8D photoperiod at 23° C. Cages were fitted with wire mesh floors and a piece of absorbent cardboard was placed underneath the wire to absorb water and urine. Voles were provided an empty metal container (9 cm diameter) for use as a nest and to minimize food and urine contamination. Each day, cakes were broken and a premeasured amount of each food was placed in separate small ceramic containers (6 cm diameter to preclude digging behavior and food spillage). Both food containers were located at the same end but opposite corner of the cage. Left or right location of the food containers within each cage was varied daily and determined randomly (coin toss). Voles were weighed daily to the nearest 0.1 g (Ohaus E400D). During the experiment, minimal food spillage occurred. After separating orts (all spilled and/or leftover food) into either lab chow or high-fat diet, they were collected and weighed daily. Separation into food types was possible because the high-fat diet was much darker in appearance due to the higher fat content. For each vole, daily food ingestion was calculated by subtracting the mass of orts from the mass of

food offered. The mean daily intake was then calculated over the duration of the experiment.

Fixed food position test

Upon completion of the random position experiment, all voles were held in individual small cages (28 x 18 x 13 cm) for two days with lab chow (Lab Diet 5001, PMI Feeds) and water *ad lib*. After this adjustment period, an additional five day experiment was conducted. We felt that if food preference was evident in the random position experiment, then the preference may be even greater if the daily selection of which food was in which position was eliminated. In the fixed position experiment, cage conditions were identical to the random position experiment, except that the food containers remained in a fixed position with the same food in each position during the entire 5 day period. Voles were given a premeasured week's supply of each type of food (approximately 140 g each). Animals were also weighed to the nearest 0.1 g (Ohaus E400F) at the beginning on the experiment and after the 5 day period. Orts (all spilled and/or leftover food) were collected, separated by food type and weighed after the five day period. For each vole, total food ingested was calculated by subtracting the mass of orts from the mass of food offered for each food type. Daily food intake was calculated by dividing the total food intake by the five days of the trial.

Statistics

Differences between average daily food intake for both experiments were analyzed using paired t-tests with a significance level set at 0.05. Since data were collected daily on the same subjects over a six day period, a repeated measures ANOVA (SAS, proc mixed) (SAS Institute, Inc., 1989) with day and food choice as fixed effects and subject as a random effect was also performed for the random position trial.

RESULTS

In the random position experiment, intake of high-fat food was 2.5 times higher (5.00 ± 0.22 g) than lab chow (1.79 ± 0.20 g) (p= 0.0001) (Table 6.3). A significant difference in food intake by individuals was also present ($F_{9,111} = 4.05$; $p = 0.0002$), the result of a wide range in total body mass (29.32 g to 70.37 g). Change in total body mass amongst subjects after six days was insignificant. At the start of the experiment, body mass averaged 56.06 ± 3.79 g and 57.34 ± 3.83 g at the end of the experiment.

In the fixed position experiment, intake of the high-fat diet (4.16 ± 0.48 g) was over five times higher than lab chow (0.80 ± 0.44 g) ($F_{1,19} = 15.56$; $p = 0.0034$) (Table 6.3). Again, the change in body mass was not significant with voles showing a body mass of 57.30 ± 3.83 g at the beginning and 56.64 ± 3.91 g after five days. In both experiments, individual voles demonstrated a consistent preference pattern with no significant variation in overall daily food intake between individuals. Overall, mean daily food intake was significantly higher in the random position experiment (6.79 ± 0.21 g) than in the latter fixed position experiment (4.96 ± 0.46 g), but the magnitude of

preference for the high-fat diet increased with the fixed position experiment (5 times greater vs. 2.5 times greater).

Repeated measures analyses of the random position experiment showed significant differences in food intake by food type ($F_{1,18} = 31.82$; $p = 0.0001$) and supports a food preference for the high-fat diet. Daily comparisons of food intake between food types were significantly different each day at p values ≤ 0.0004 (Figure 6.1), showing a strong preference for the high-fat diet. Within a specific food type, general patterns were evident. For lab chow, daily intake did not vary significantly over the 5 day period and ranged from 1.38 ± 0.51 to 1.91 ± 0.51 g/day. The daily intake of the high-fat diet was also very consistent (4.32 ± 0.41 to 4.94 ± 0.41 g/day), except for a significantly higher intake in day three (6.31 ± 0.51 g/day). Overall, the preference for the high-fat diet did not strengthen over time, but was strong from day 1 through day 6 (Figure 6.1). For each day in both tests, voles chose the high-fat diet in a consistent and convincing manner.

DISCUSSION

Although it is often concluded that meadow voles consume a relatively low quality diet consisting mainly of grasses and sedges, results of our food preference studies suggest that voles can preferentially select foods of high-fat content. In this study, voles preferred the high-fat diet, almost to the exclusion of lab chow. Since the lab reared voles used in this study were fed exclusively lab chow after weaning, we cannot rule out the effects of a novel food on their preference behavior. However, by preparing both food choices in a similar fashion, we attempted to eliminate novel food concerns.

Previous investigators have studied effects of fiber, protein, and essential minerals on forage choice in voles (Bergeron and Jodoin 1987, 1991; Bucyanyandi and Bergeron 1990; Christian 1989; Ferkin et al. 1997; Harju and Hakkarainen 1997; Keys and VanSoest 1970; Mickelson and Christian 1991). Keys and VanSoest (1970) demonstrated that voles will select less fibrous sections of the plant leaves and heads over the courser stalks. When given a choice between foods with varying levels of protein, voles selected foods with higher protein content (Harju and Hakkarainen (1997). Bergeron and Jodoin (1987) suggest that voles choose foods with high protein content and low levels of digestive inhibitors and not on the basis of caloric content or availability. In addition, voles fed a high-protein diet (25 % vs. 15 % protein content) produced odors which were preferred by potential female partners (Ferkin et al. 1997). Mickelson and Christian (1991) showed that captive meadow voles have the ability to discriminate among diets on the basis of potassium content, and that they avoid diets high in potassium. Christian (1989) concluded that potassium loading has no effect on sodium balance in meadow voles and that they possess a well developed physiological ability to handle excess potassium loads. In general, meadow voles select food of lower fiber, higher protein, and adequate levels of potassium and sodium. Rarely has available dietary fat been addressed or inferred in studies of forage in non-hibernating vegetation feeding small mammals.

Since a high preference for the high-fat diet was shown in the random position experiment, we expected and found a stronger preference in the fixed position experiment. Voles ate 5 times more of the high-fat diet when the food position was fixed compared to 2.5 times more of the high-fat diet when voles had to search and sample food from

random positions. Interestingly, voles had lower daily food intake in the fixed position trial (4.96 ± 0.46 g) than in the random position trial (6.79 ± 0.21 g). One possible explanation for the reduced intake may be a "learned response" in which voles chose a similar forage pattern each day. Also, the elimination of food sampling and selection during the fixed position experiment may be the cause for lower overall intake.

In Table 6.2, the high-fat diet had lower levels of both protein and critical minerals compared to lab chow. Thus, if voles select for minerals, they should have eaten more lab chow with higher mineral concentration. Similarly, the higher protein concentration in lab chow should also make lab chow the food choice if voles select for protein. However, in all cases, voles selected the high-fat diet over lab chow. Potentially, voles could have eaten more of the high-fat diet to ensure intake of adequate dietary protein and minerals. In contrast, they could have chosen lab chow, which contained higher levels of both protein and minerals, but they did not. We believe that the protein and mineral levels in the high-fat diet were adequate and that the food choice of voles in this study was associated with higher levels of fat in the high-fat diet.

From our results, we are not inferring that meadow voles consume a high-fat diet in the field. However, since many microtines are relatively lean in the wild, their body composition may be the result of a low quality diet with low dietary fat. We have shown that meadow voles prefer a high-fat food over lab chow when given a choice, and therefore, can differentiate dietary fat in foods. Whether the diet of meadow voles actually contains more fat than previously documented may be a possibility. During periods of the

year when plant possess higher dietary fat in certain reproductive structures (heads, seeds), meadow voles may select these parts as a greater percentage of their diet.

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Table 6.1. Percent body fat of wild, non-hibernating small mammals.

Species	Fat Content % Total Body Mass	Reference
Meadow vole (*Microtus pennsylvanicus*)	3 - 7	1
	3 - 8	9
Pine vole (*Microtus pinetorum*)	5 - 7	5
Prairie vole (*Microtus ochrogaster*)	4 - 5	6
	3 - 5	8
Red-backed voles (*Clethrionomys gapperi*)	3 - 5	1
Brown lemming (*Lemmus sibiricus*)	3 - 5	2
Deer mouse (*Peromyscus maniculatus*)	3 - 5	7
	5 - 7	3, 6
White-footed mouse (*Peromyscus leucopus*)	5 - 8	4
Harvest Mouse (*Reithrodontomys megalotis*)	3 - 5	7
	6 - 7	3
Grasshopper mouse (*Onychomys leucogaster*)	4 - 6	7

1. Anderson and Rauch 1984
2. Batzli and Esseks 1992
3. Fleharty et al. 1973
4. Lynch 1973
5. Lochmiller et al. 1983
6. Morton and Lewis 1980
7. Schreiber and Johnson 1975
8. Voltura 1997
9. This study

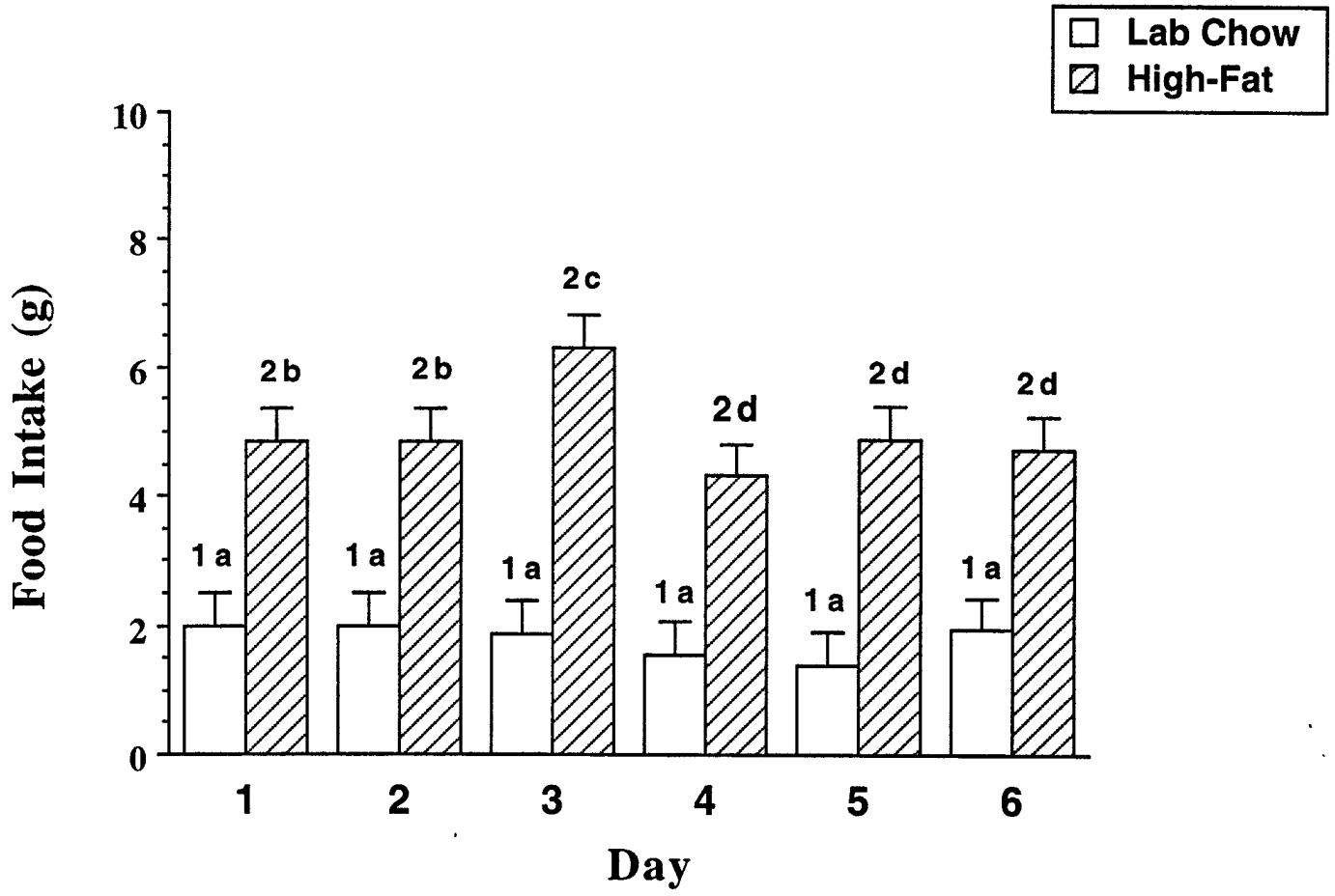
Table 6.2. Proximate analysis of food types used in food preference experiment. One cake per food type was analyzed. Column values represent composition as received (A). Cakes were further dried to constant mass by the analysis lab and reported on a dry matter basis (B).

	Lab Chow		Lab Chow + Fat	
	A	B	A	B
%				
Moisture	15.67	0.00	16.41	0.00
Dry Matter	84.33	100.00	83.59	100.00
Crude Protein	22.65	26.97	16.11	19.18
ADF	5.85	6.73	5.50	6.55
Nitrogen	3.62	4.31	2.58	3.07
Phosphorous	0.61	0.73	0.41	0.49
Potassium	1.18	1.40	0.95	1.13
Calcium	0.92	1.10	0.61	0.73
Magnesium	0.19	0.23	0.14	0.17
Sodium	4.07	4.85	2.69	3.20
mg/kg (ppm)				
Iron	234.9	279.60	176.4	210.0
Manganese	64.87	77.23	42.05	50.06
Zinc	79.90	95.12	52.05	61.96
Copper	14.11	16.80	10.14	12.07
Boron	9.64	11.48	7.52	8.95
Molybdenum	0.96	1.14	1.13	1.35

Table 6.3. Diet choice by meadow voles (*Microtus pennsylvanicus*) given a choice of lab chow (5 % fat) or lab chow + fat (25 % fat) (n=10 per group). Values given are mean plus/minus one standard error (in parentheses). Different letters indicate significant differences between food choices (paired t test, $p \leq 0.05$). Comparisons are within a particular study and not between studies.

	Intake (g/d)
Random position experiment	6.79 (0.21)
Lab Chow	1.79 (0.20)[A]
Lab Chow + Fat	5.00 (0.22)[B]
Fixed position experiment	4.96 (0.46)
Lab Chow	0.80 (0.44)[A]
Lab Chow + Fat	4.16 (0.48)[B]

Figure 6.1. Comparison of daily food intake of each food type for voles given a food choice during the random position experiment. Values are mean plus/minus one standard error (error bars). Different numbers indicate a significant difference between food type consumption each day ($p < .05$). Different letters indicate a significant difference in food intake on consecutive days within a particular food type ($p < .05$).



Chapter 7

Effects of activity on body composition of wild meadow voles maintained under laboratory conditions

INTRODUCTION

The body fat of small non-hibernating mammals in the wild ranges from 3 to 9 % of total body mass (Table 7.1). In addition, body fat may vary in response to nutritional conditions or as a physiological response to environmental cues in preparation for coming events (Batzli and Pitelka 1971; Batzli and Esseks 1992; Didow and Hayward 1969). The rates of body fat deposition and catabolism both in the wild and in the lab have also been linked to environmental factors such as temperature, photoperiod, food quality, food intake, and activity (Batzli and Esseks 1992; Cengel et al. 1978; Donald et al. 1980; Ferns and Adams 1974; Holleman and Dieterich 1978; Millar 1981; Nagy and Negus 1993; Nagy et al. 1994; Rock and Williams 1979; Sawicka-Kapusta 1970, 1974; Voltura 1997).

Small mammals maintained in the laboratory are known to deposit large amounts of fat (Batzli and Esseks 1992; Donald et al. 1980; Ferns and Adams 1974; Voltura and Wunder 1998). Such fattening may be due to unnatural, high quality diets, stable warm environmental conditions which decrease thermoregulatory requirements (Wunder 1984, 1985), or cage confinement leading to decreased activity. In our lab, when meadow voles were removed from the wild and held under stable laboratory conditions with lab chow (Lab Diet 5001, PMI Feeds) or rabbit chow (Lab Diet 5325, PMI Feeds), body fat rose from 5 to 25 % over a six week period (Chapter 5). During these experiments, we did not measure vole activity and assumed that activity was minimal when voles were maintained

in individual cages (28 x 18 x 13 cm). As a result, we felt it necessary to investigate the effects of activity on the changes in body composition we observed.

The literature contains conflicting statements about the 24 hour activity pattern in meadow voles. Some observers have found meadow voles active at all times (Hamilton 1937a, 1937b; Hatt 1930), while others report primarily a diurnal pattern (Emlen et al. 1957). Later studies have shown predominantly nocturnal activity with smaller diurnal pulses in activity (Dewsbury 1980; Madison 1985; McShea and Madison 1984). In addition, when held within a lab, activity patterns, timing and duration may change (Davis 1933; Hatfield 1940; Morrison 1948). Madison (1985) reported that Graham (1968) showed diurnal activity in free-ranging meadow voles, but caged individuals were crepuscular, and caged voles in the laboratory were nocturnal.

Although microtine rodents may have different behavioral and physiologic rhythms, a 2-4 hour ultradian activity rhythm is a common feature for many vole species (Hatfield 1940; Madison 1985; Wiegert 1961). Such a rhythm is suggested to be essentially a feeding rhythm and its periodicity is linked closely to energy needs, ambient temperature and food quality (Hatfield 1940; Madison 1985; Wiegert 1961). The herbivorous diet of *Microtus*, consisting of large amounts of low quality food (Batzli 1985; Bergeron and Jodoin 1987), necessitates frequent feeding through the 24 hour cycle with rest periods for efficient digestive processing. The result is an ultradian rhythm which entails 1 to 3 hours of rest followed by 1 to 3 hours of activity and is inversely related to energy demand. The period length of the ultradian rhythm has been shown to be directly proportional to body weight, and inversely proportional to metabolic mass (Daan

and Aschoff 1981). Thus, the smaller the vole, the greater the energy needs per gram of body mass (Kleiber 1947), and the shorter will be the length of the ultradian rhythm. Overall, short-term activity in microtines reveal general patterns which include: 1) the period varies within and between individuals; 2) activity pulses are more prominent at certain times of the 24 hour cycle; 3) activity peaks shift with season; and 4) activity peaks appear to synchronize with dawn and dusk (Madison 1985).

Since activity patterns seem to be influenced by energy demands, one might expect lesser activity in generalist herbivores, especially when the demand for food is low and food resources are plentiful. During food abundant periods, the time necessary to search for acceptable foods should be minimal and the energy expended in foraging may be reduced. In contrast, winter conditions bring decreased food availability and quality, as the proportion of nitrogen and soluble carbohydrates drops relative to the fiber content of the forage (VanSoest 1994; Wunder et al. 1977). However, whether activity is truly affected by these conditions remains unanswered. Nevertheless, if the need to search for food is minimal, combined with increased predator avoidance if inactive, it seems reasonable that remaining in the nest may be advantageous in the nonbreeding season. Such a lifestyle is contradictory to the common perception that many small mammals are highly active. However, whether daily activity and associated energy requirements are influential in the overall body condition in small mammals have not been demonstrated. Therefore, we undertook this study to determine whether voles held in the lab (which become abnormally fat) with access to activity would remain more lean than those which are more sedentary.

MATERIALS AND METHODS

Meadow voles were trapped using Sherman livetraps (28 x 18 x 13 cm) from local populations in three mixed-grass riparian fields at the United States Air Force Academy, El Paso County, Colorado, in October 1997. A total of 16 adult male voles (> 29 g) were collected.

Captured voles were immediately given an apple slice and piece of lab chow (Lab Diet 5001, PMI Feeds) in order for rehydration and to provide gut-fill. They were maintained within capture traps and transported to a laboratory at the United States Air Force Academy to estimate body composition using EM-SCAN® procedures as described by Voltura and Wunder (1998). Measurements of body composition were completed within 2 to 3 hours after collection. Voles were then transferred to Colorado State University, Fort Collins, CO, the following day and maintained in an environmental chamber in individual small cages (28 x 18 x 13 cm) under a photoperiod condition of 11L:13D at 23° C for one week. During the first week of capture, two voles died. After one week, body composition was again measured. Voles were weighed to the nearest 0.01 g (Ohaus EF400D) and ranked by mass. Then, they were assigned alternately to either a control or experimental group to ensure no statistical difference between groups for body mass. One vole in the experimental group also died during week 3 and was removed from the data set

Control voles were housed individually in opaque mouse cages (28 x 18 x 13 cm), while experimental voles were housed in specially constructed cages of similar size but containing running wheels. Although the cages were different between groups, we felt they were similar enough in size and design to not be a source of variation. To measure individual vole activity, each rotation of the running wheel closed a switch, which was recorded by an event recorder and counter. The counter was read daily and values summed weekly for each vole. The duration of the experiment was six weeks with body composition measured weekly. In addition, all voles received a weekly premeasured amount of pelleted lab chow (Lab Diet 5001, PMI Feeds) (approximately 100 g) in a metal container with lid to preclude food spillage. Although food was checked daily and added when necessary, orts were removed only after seven days and dried for 24 hours at 60° C. Orts were then weighed and the difference between the food given and orts determined weekly food intake for each vole. Mean daily intake by group was then calculated.

Statistics

A repeated measures ANOVA (SAS, proc mixed) with time (week) and treatment (activity, control) as the fixed effects and subject nested within treatment as a random effect was performed (SAS Institute, Inc. 1989). Additional comparisons between groups for each body composition parameter were also completed with regard to treatment and time. The activity level for individuals within the control group was assumed to be zero for the entire experimental period. We realize that voles could move about in their cages but they could not run continuously unless they did so in circles which we did not observe.

In addition, within the activity group, the relationship between level of activity and change in body composition was investigated using regression techniques. Models for each body composition parameter were evaluated with activity (revolutions per time period/1000) as the independent variable and body composition change (Δg per time period) as the dependent variable. Body composition change accounted for the following time periods: start to week 2; week 2 to week 4; week 4 to week 6; and start to week 6. For all comparisons, statistical significance was set at $p < 0.05$.

RESULTS

Body composition change associated with activity

Within each group, there was significant variation over time for both total body mass ($F_{6,66} = 14.41$; $p = 0.0001$) and fat-free mass ($F_{6,66} = 5.34$; $p = 0.0002$) (Figure 7.1, 7.2), but no significant difference between treatments was found for these parameters at the end of six weeks. There was a significant time by activity interaction for lipid mass ($F_{6,66} = 5.17$; $p = 0.0002$). Specifically, lipid mass increases after six weeks were significantly greater for the activity group (12 g) than the control group (10 g) (Figure 7.3). Such results are contrary to what we expected. A significant time by activity interaction was also present for percent lipid mass ($F_{6,66} = 5.96$; $p = 0.0002$), reflecting the unequal change in lipid mass between treatments (Figure 7.4).

At the end of six weeks, both groups showed significant change in total body mass, lipid mass, and fat-free mass within each group over time ($p \leq 0.01$). The activity group increased body mass 7.11 ± 1.47 g with a gain in lipid mass of 11.78 ± 0.73 g and a loss of

4.67 ± 1.25 g of fat-free mass over six weeks. In the control group, voles gained 6.64 ± 1.58 g of body mass due to increases in lipid mass of 10.00 ± 0.79 g and a reduction in fat-free mass of 3.37 ± 1.35 g over the same period. Between group comparisons by week did not show significant differences for total body mass or fat-free mass. Both groups increased body mass by approximately 7 g while losing 3 to 4 g of fat-free mass over the six week period (Figure 7.5, 7.6). In addition, both groups deposited lipid mass in a similar fashion with large gains in lipid mass from the start of the experiment through week 3. From week 3 forward, the activity group had greater lipid mass in weeks 4 and 6 than the control group (Figure 7.3, 7.4). These results were contrary to our expectations, with the activity group depositing more lipid mass and having a higher percent lipid mass after six weeks than the group in the control cages.

Within the activity group, the number of wheel revolutions each two week period were greater than the previous period. The circumference of the running wheel was 53 cm. During the first two week period, wheel revolutions averaged 1610 ± 910 per day equating to approximately 0.85 kilometers per day. From week 2 to week 4, average wheel revolutions were 2203 ± 746 per day or 1.17 kilometers per day. During the final two week period, activity levels increased to 2229 ± 595 revolutions per day or 1.18 kilometers per day. Over the duration of the experiment, voles in the activity group averaged 1.07 ± 0.44 kilometers of running per day.

For food intake, a significant treatment by week interaction was present ($F_{5,55}$ = 6.84; p = 0.0001). This was the result of significant differences between groups during the initial two weeks of the experiment. During these weeks, the activity group consumed 5.

43 ± 0.36 g of food per day while the control group ate 6.82 ± 0.44 g per day on week 1 and 7.08 ± 0.54 g per day on week 2. However, after this period, there were no significant differences between groups for food intake for the remainder of the experiment (Figure 7.7). Within the activity group, food intake did not vary significantly from week to week over the entire experiment. The control group had significant differences in intake from week 2 to week 3, but did not vary from that time forward. (Figure 7.7). These results did not support increased food intake as we expected associated with greater energy expenditure from activity.

Relationship between level of activity and body composition change

As seen in Table 7.2, the relationship between level of activity and change in body composition was not predictive. With the exception of changes in total body mass and fat-free mass from week 2 to 4, all other models had slopes which did not differ from zero, combined with very small r-squared values. Models illustrating the relationship between the change in each body composition parameter versus overall activity are presented in Figure 7.8 - 7.10. Viewing these results and the comparison with control responses, there is no relationship between activity and fat deposition.

DISCUSSION

In contrast to our expectations, activity did not result in a leaner body composition. Our results showed larger fat deposition by experimental versus control animals. Changes in body composition within the control group were similar to those reported in previous studies of various small mammals where activity was not measured (Batzli and Esseks 1992; Donald et al. 1980; Ferns and Adams 1974; Voltura and Wunder 1998). Over the six week period, voles in the control group gained 10 g of lipid mass resulting in a rise in percent body fat from 2.6 % to 24.8 %. Voles with access to running wheels did not reduce fat gain. Voles which ran an average of 1.07 ± 0.44 kilometers per day over six weeks still gained an average of 12 g of lipid mass (Figure 7.3) and increased percent body fat to 30.5 % (Figure 7.4). In both groups, changes in total body mass were due primarily to large increases in lipid mass combined with smaller losses in fat-free mass. As seen in Figure 7.5, changes in total body mass was very similar between groups, as were changes in fat-free mass (Figure 7.6). These results may be explained in several ways.

If an animal is not physiologically stressed, activity may not affect body composition in meadow voles. Since environmental conditions were stable and warm in our experiment and food was both abundant and of high quality, any effects on body composition due to activity may have been masked by the influence of these factors. In addition, we cannot ensure that the 1.07 ± 0.44 kilometers per day is truly representative of activity levels in the field. Since we did not measure activity in the control and assumed that their activity level was zero, this may be an incorrect assumption. Control voles were

not hindered from moving and there was adequate space within an individual cage to allow activity. However, we did not see the control animals continually moving about their cages nor did we observe any unusual activity (pacing, circular patterns of running) by control animals. In most cases, control voles were either feeding, preening, or resting. Thus, we feel that assuming no activity in the control condition was not unjustified.

Whether the measured level of effort was representative of the activity level of wild voles is unknown. It is very difficult to observe and measure activity of wild voles since they spend most of their time out of sight under the grass overstory. Data collected on microtine home ranges using radio telemetry, fluorescent powdertracking, and trapping can be helpful, but large intraspecific variability associated with sex and season often exists. Males may expand or shift home ranges with the occurrence of estrus in neighboring females (Madison 1985). Females may contract daily range at parturition and expand during weaning (Madison 1978). In addition, large variation in home range size has also been reported. Using radiotelemetry, Madison (1980) found that male meadow voles had larger home ranges (192 m^2) than females (68 m^2) in summer. In contrast, using live trapping, Blair (1940) reported much larger home ranges in spring and summer for both males (1619 m^2) and females (1012 m^2). Another limitation of home range data is that they often do not include when and to what extent activity occurs. As a consequence, we allowed voles to be active when they wanted to be active and measured this level of effort on a daily basis with an event recorder. With our experimental design, we acknowledge a potential for bias in activity data. Voles may be active purely out of boredom or "capture stress" and result in higher than normal expenditure. In contrast,

voles may react to a new environment by becoming inactive which results in lower than normal effort. However, as seen in Figure 7.8 - 7.10, only one vole exhibited any large degree of variation in activity from the group.

The relationship between level of effort and change in all body composition parameters was not predictive. Since the greatest change in body composition over six weeks was in lipid mass, we thought that this change could be explained by level of activity, but our results were unsupportive. Voles with measured activity demonstrated large changes in body composition, unrelated to level of activity. We interpret these results to further support the conclusion that activity does not strongly influence body composition in meadow voles. These results are contradictory to previous reports in which increased activity resulted in decreased body fat (Batzli and Esseks 1992; Bell et al. 1997; Cortright et al. 1997; Kortner and Geiser 1995). Batzli and Esseks (1992) showed that brown lemmings with access to running wheels had considerably less fat than control animals (20.3 % vs. 33.5 %), but were still much fatter than lemmings captured in the field. In addition, lemmings with wheels lost 1.7 g of body mass compared to a gain of 5.5 g in the control. In white mice, body fat was reduced with exercise regardless of diet which varied in dietary fat (beef fat, 12.6 ; low fat, 7.4; canola oil, 9.6 g/100 g body mass; Bell et al. 1997). Cortright et al. (1997) also demonstrated that lab rats decreased body fat (14.6 % to 8.0 %) when exercised for 9 weeks. In all these studies, food intake also increased directly with level of activity.

In our study, meadow voles removed from the field changed body composition similarly, regardless of activity. Voles with running wheels ran an average of 1.07

kilometers per day and had higher lipid deposition and nearly identical changes in body mass and fat-free mass to control animals. These results suggest that activity may not strongly influence the lean body composition observed in field-caught meadow voles.

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Table 7.1. Fat content of wild small mammals. Reported as percent body fat.

Species	Fat Content	Reference
Meadow vole (*Microtus pennsylvanicus*)	3 - 7	1
	3 - 8	10
Pine vole (*Microtus pinetorum*)	5 - 7	6
Prairie vole (*Microtus ochrogaster*)	4 - 5	7
	3 - 5	9
	4 - 6	2
Red-backed voles (*Clethrionomys gapperi*)	3 - 5	1
Brown lemming (*Lemmus sibiricus*)	3 - 5	3
Deer mouse (*Peromyscus maniculatus*)	3 - 5	8
	5 - 7	4, 7
White-footed mouse (*Peromyscus leucopus*)	5 - 8	5
Harvest Mouse (*Reithrodontomys megalotis*)	3 - 5	8
	6 - 7	4
Grasshopper mouse (*Onychomys leucogaster*)	4 - 6	8

1. Anderson and Rauch 1984
2. Baker 1971
3. Batzli and Esseks 1992
4. Fleharty et al. 1973
5. Lynch 1973
6. Lochmiller et al. 1983
7. Morton and Lewis 1980
8. Schreiber and Johnson 1975
9. Voltura 1997
10. This study

Table 7.2. Summary of regression equations evaluating the relationship between the level of activity on change in body composition parameters over three consecutive two week periods. y = body composition parameter; x = level of activity (# of revolutions/1000)

Condition	Δ g ± 1 se	Equation	r squared	p Value
••Total body mass change (g)				
Start to Week 2	-0.14 (1.57)	y = -.0939 x + 1.9799	0.5802	0.0466
Week 2 to Week 4	5.92 (1.41)	y = .1064 x + 2.6433	0.6232	0.0348
Week 4 to Week 6	1.32 (0.87)	y = -.0050 x + 1.7027	0.0853	0.5250
Start to Week 6	7.10 (1.08)	y = .0025 x + 6.819	0.0049	0.8818
••Lipid mass change (g)				
Week 0 to Week 2	3.59 (0.86)	y = -.0183 x + 3.9982	0.0740	0.5552
Week 2 to Week 4	6.05 (0.76)	y = .0400 x + 4.8127	0.3052	0.1985
Week 4 to Week 6	2.14 (0.60)	y = -.0047 x + 2.5068	0.1619	0.3708
Start to Week 6	11.77 (1.20)	y = .0109 x + 10.849	0.0751	0.5521
••Fat-free mass change (g)				
Week 0 to Week 2	-3.72 (1.38)	y = -.0756 x - 2.0184	0.4882	0.0807
Week 2 to Week 4	-0.12 (0.82)	y = .0664 x - 2.1694	0.7095	0.0174
Week 4 to Week 6	-0.82 (0.43)	y = -.0003 x - 0.7987	0.0012	0.9402
Start to Week 6	-4.67 (0.80)	y = -.0084 x - 3.952	0.1013	0.4867

Figure 7.1. Body composition of voles over a six week period with running wheels. Values are mean plus/minus one standard error (error bars) Different letters indicate significant differences between consecutive weeks for each body composition parameter ($p < .05$). * indicates significant difference from parameter value at the start of the experiment ($p < .05$).

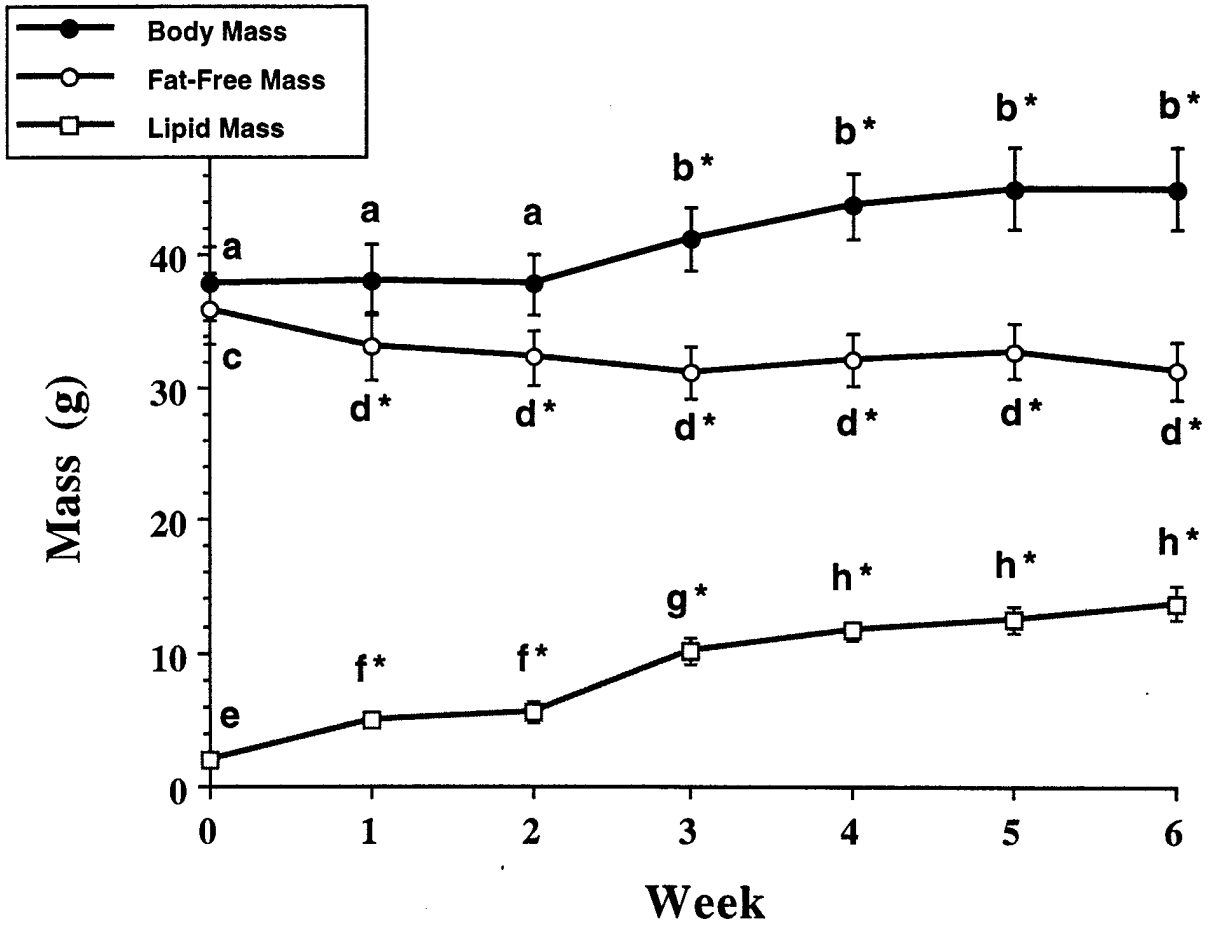


Figure 7.2. Change in body composition of voles over a six week period without running wheels. Values are mean plus/minus one standard error (error bars) Different letters indicate significant differences between consecutive weeks for each body composition parameter ($p < .05$). * indicates significant difference from parameter value at the start of the experiment ($p < .05$).

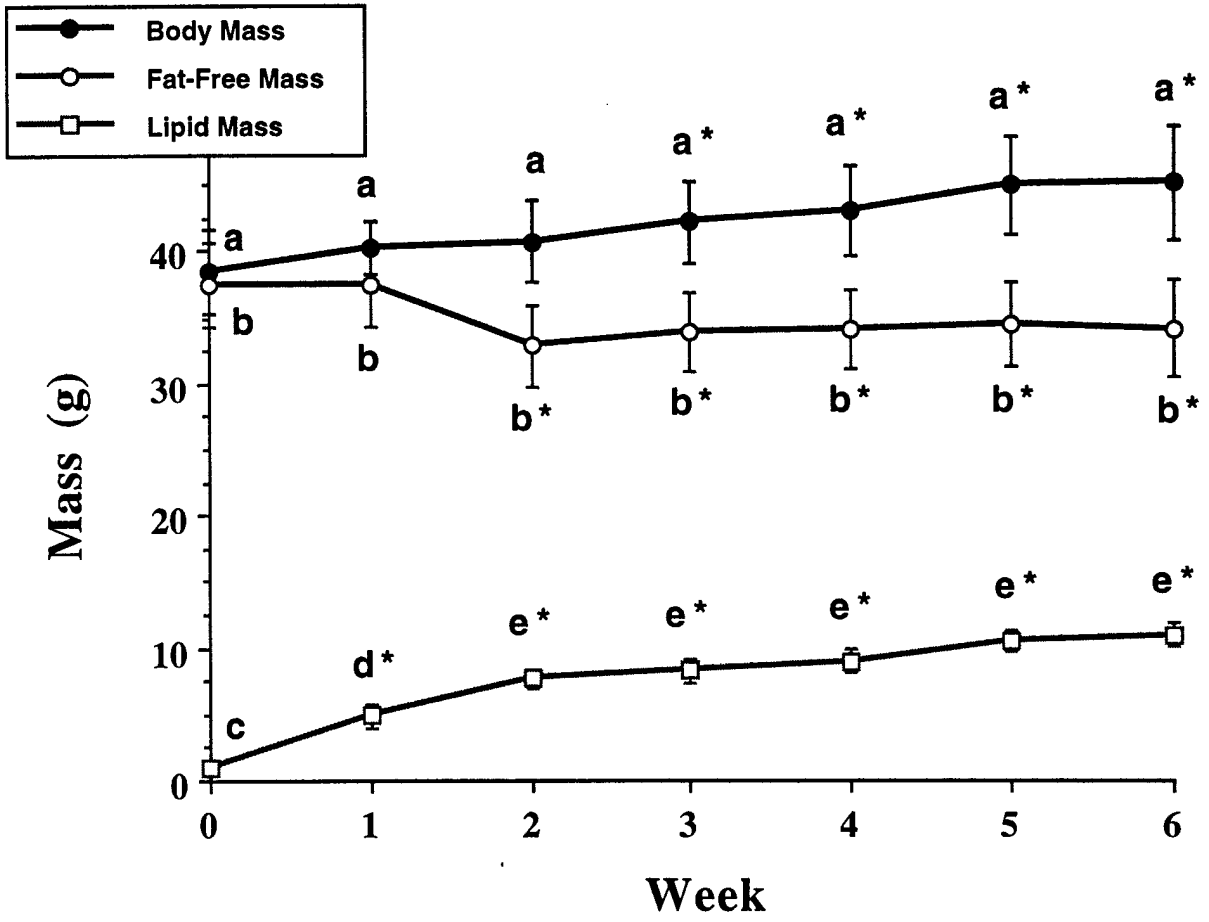


Figure 7.3 Comparison of lipid mass between voles with running wheels (activity) and controls. Values are mean plus/minus one standard error. Different letters indicate significant differences between groups ($p < .05$). Different numbers indicate significant differences in consecutive week to week comparisons within each group ($p < .05$).

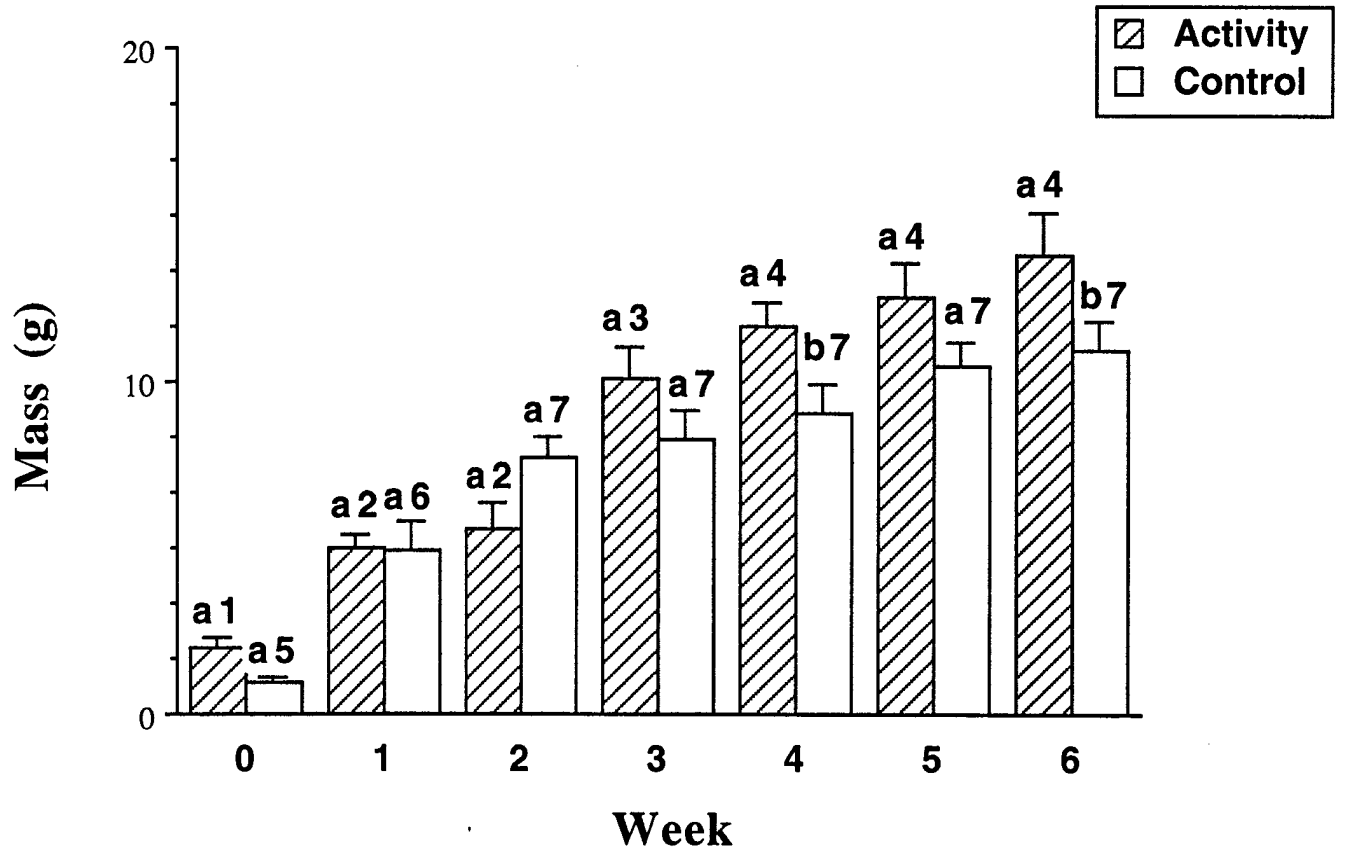


Figure 7.4. Comparison of percent body fat between voles with running wheels (activity) and controls. Values are mean plus/minus one standard error. Different letters indicate significant differences between groups ($p < .05$). Different numbers indicate significant differences in consecutive week to week comparisons within each group ($p < .05$).

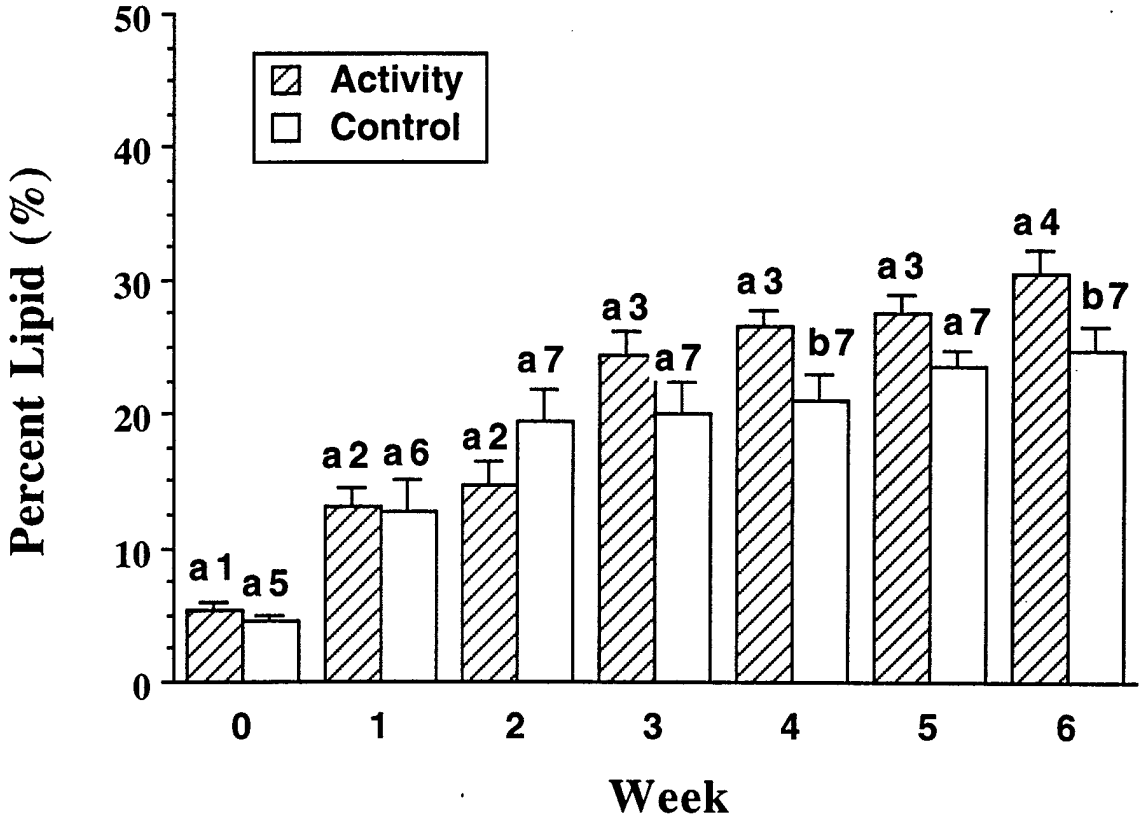


Figure 7.5. Comparison of total body mass between voles with running wheels and controls. Values are mean plus/minus one standard error. Different letters indicate significant differences between groups ($p < .05$). Different numbers indicate significant differences in consecutive week to week comparisons within each group ($p < .05$).

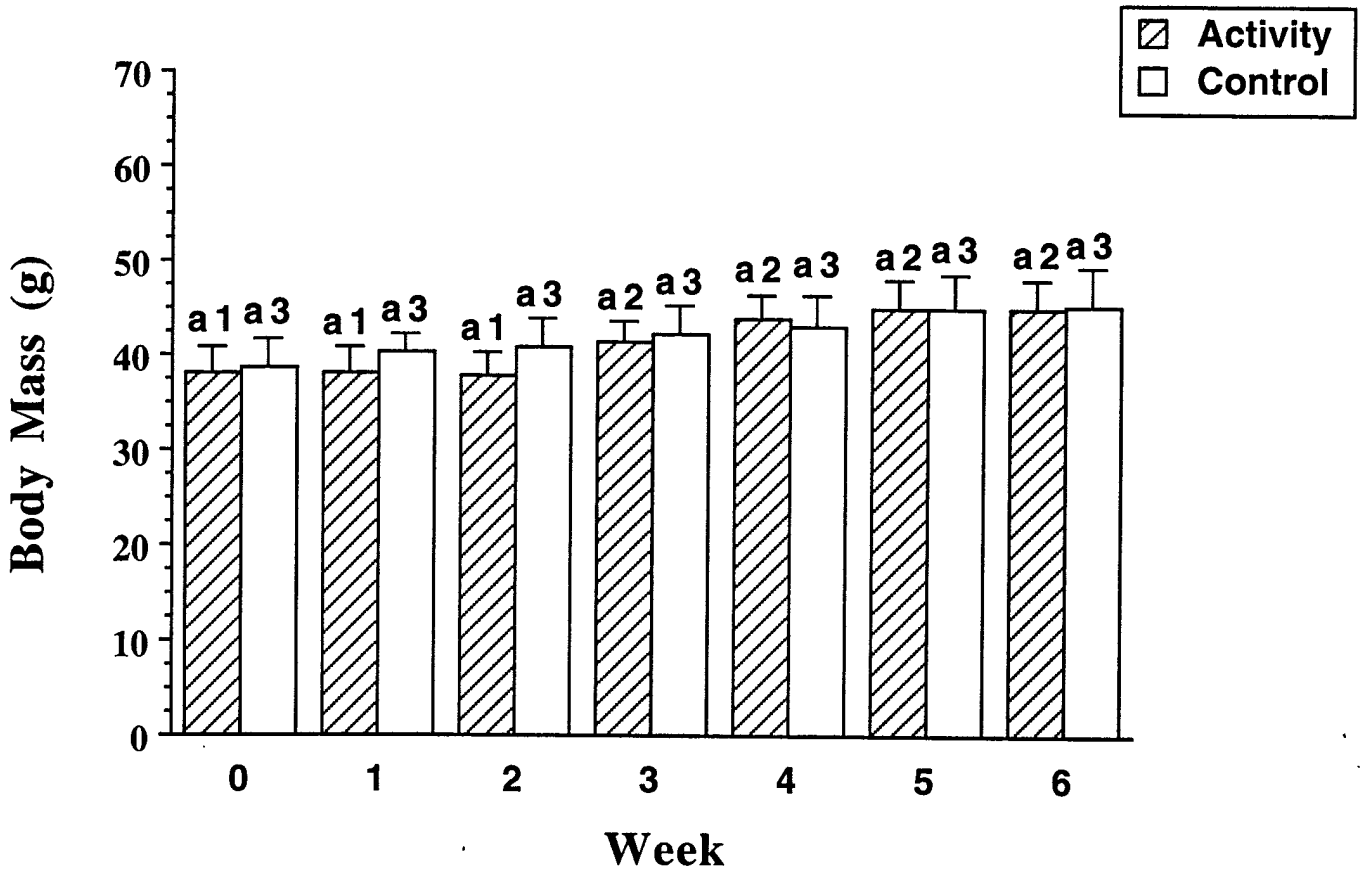


Figure 7.6. Comparison of fat-free mass between voles with running wheels and controls. Values are mean plus/minus one standard error. Different letters indicate significant differences between groups ($p < .05$). Different numbers indicate significant differences in consecutive week to week comparisons within each group ($p < .05$).

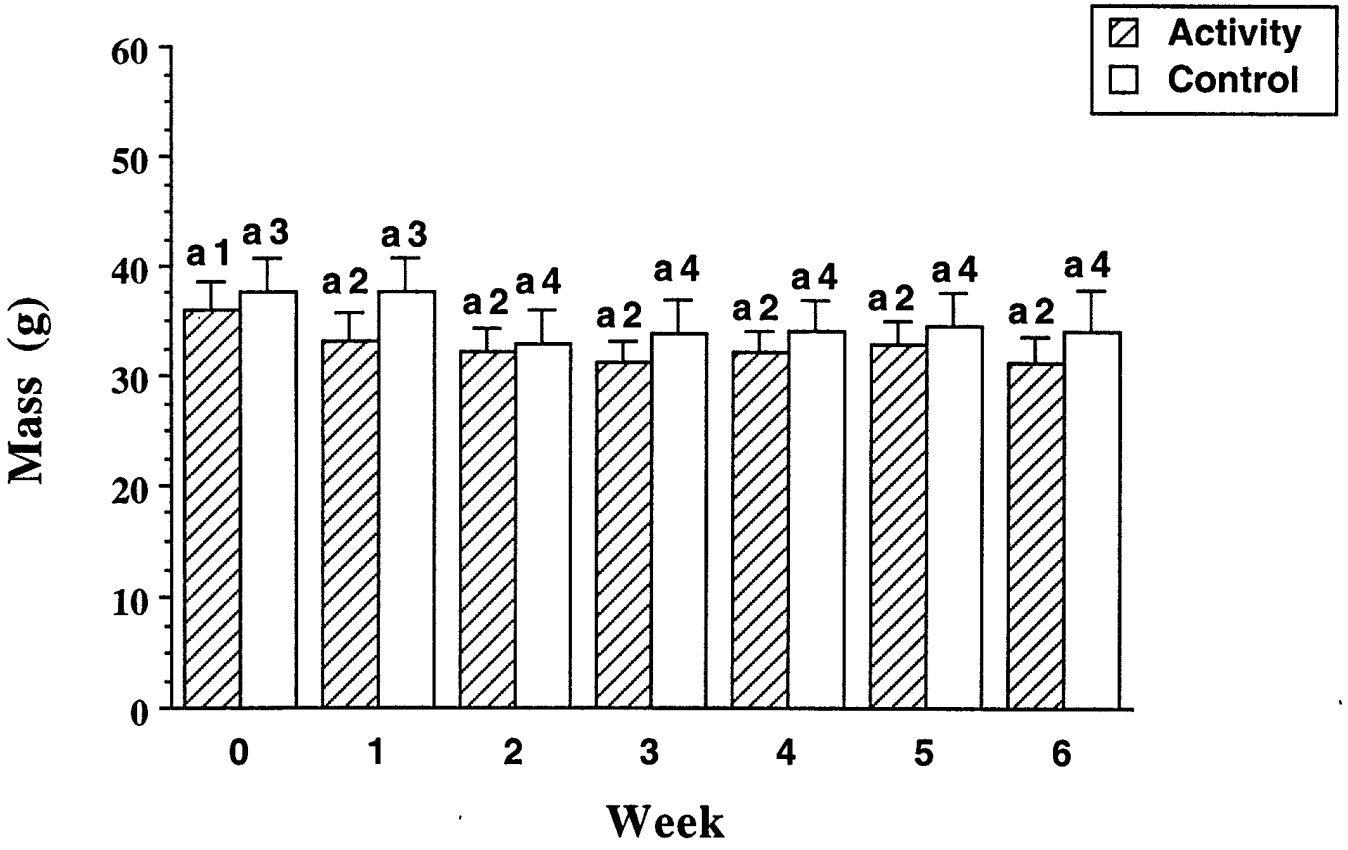


Figure 7.7. Comparison of daily food intake between voles with running wheels (activity) and controls. Values are mean plus/minus one standard error (error bars). Different letters indicate significant differences between groups ($p < .05$). Different numbers indicate significant differences in consecutive week to week comparisons within each group ($p < .05$).

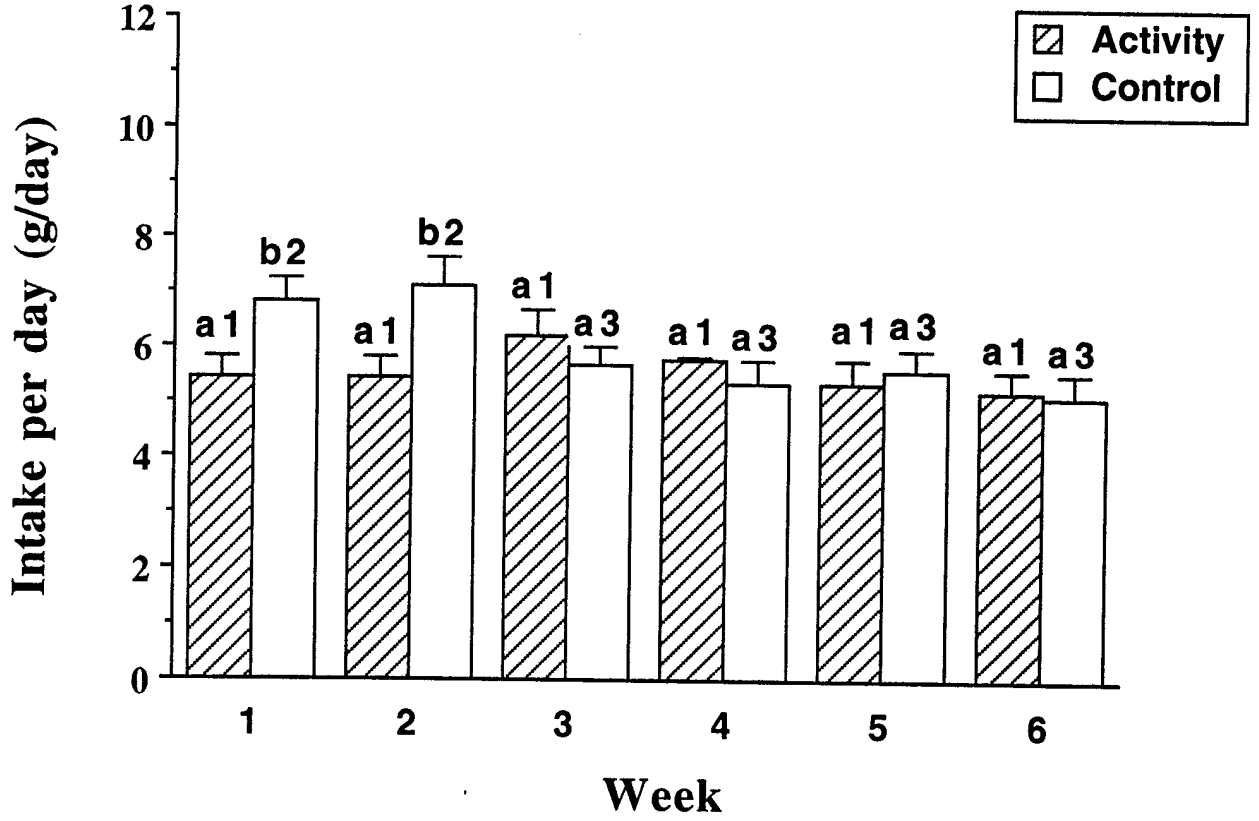


Figure 7.8. Relationship between the change in lipid mass and activity level for voles on running wheels for six weeks. Slope of regression line was not statistically different from 0 ($p = .5521$). y = change in lipid mass; x = activity (revolutions/1000)

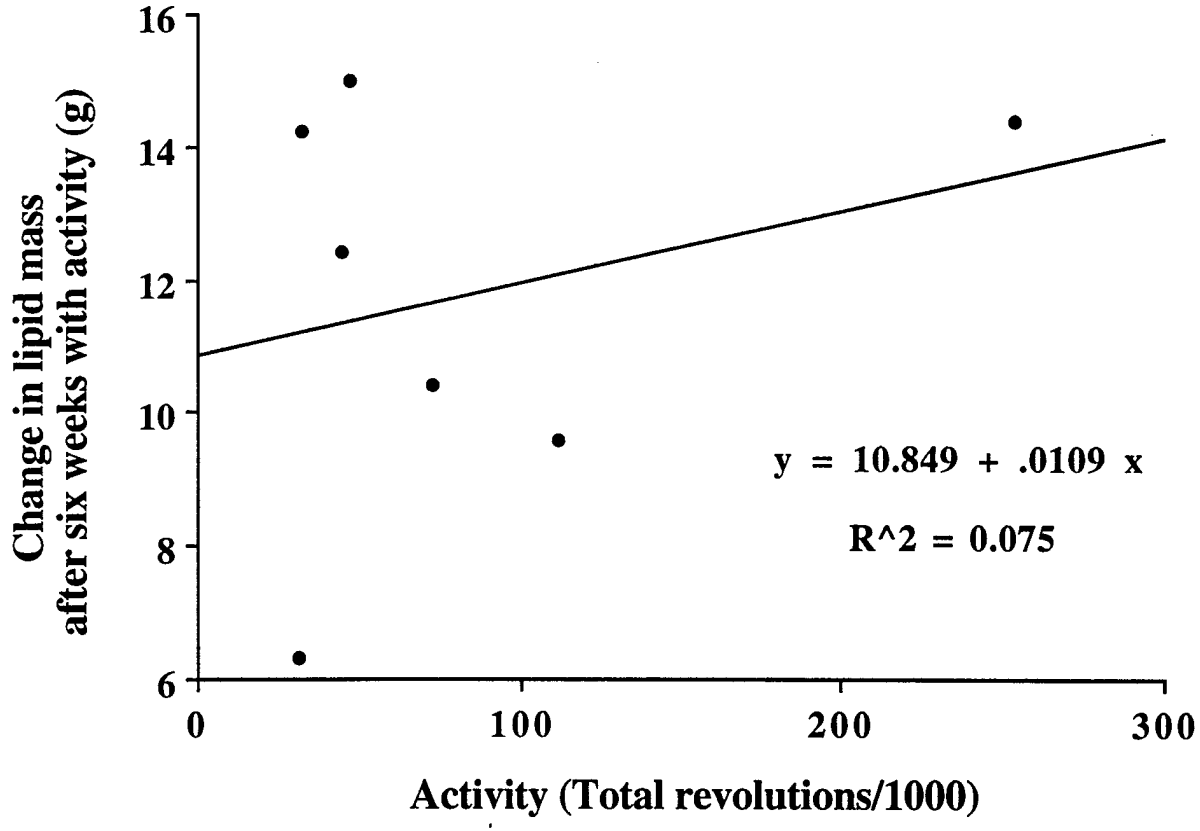


Figure 7.9. Relationship between the change in body mass and activity level for voles on running wheels for six weeks. Slope of regression line was not statistically different from 0 (p = .8818). y = change in total body mass; x = activity (revolutions/1000)

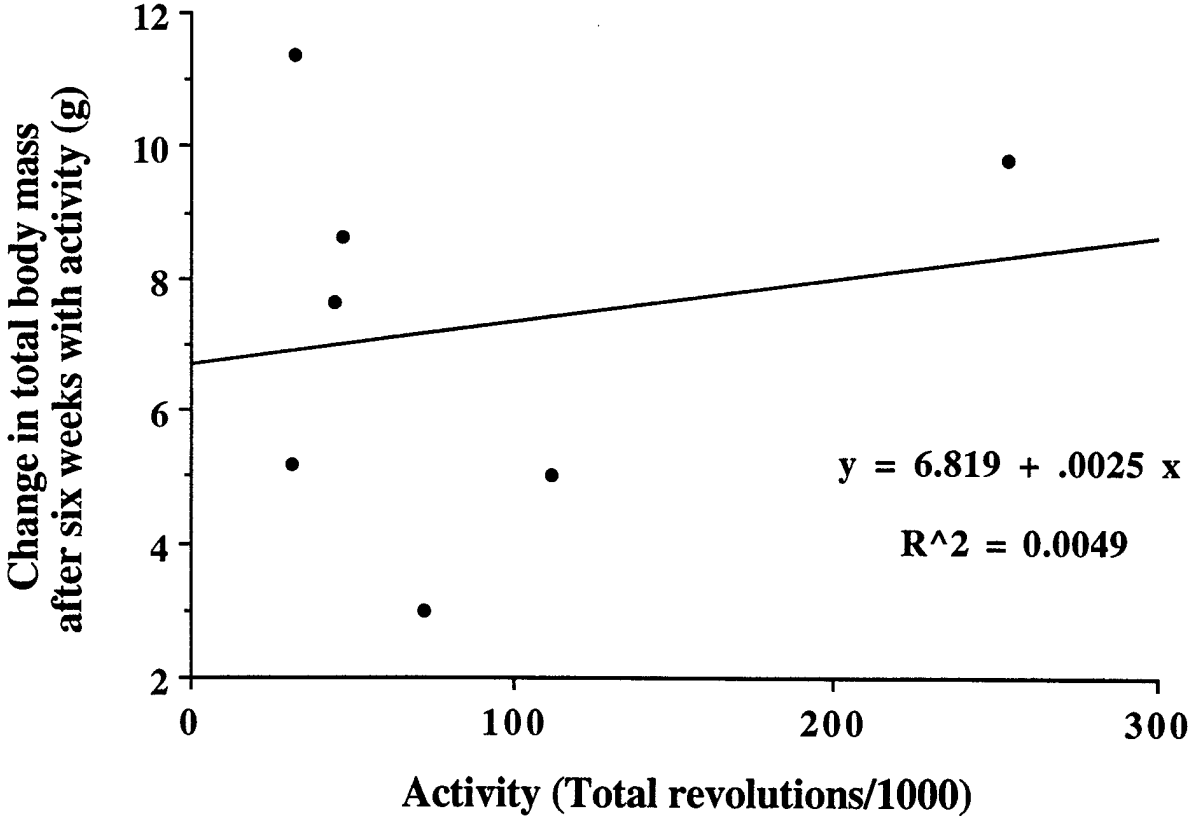
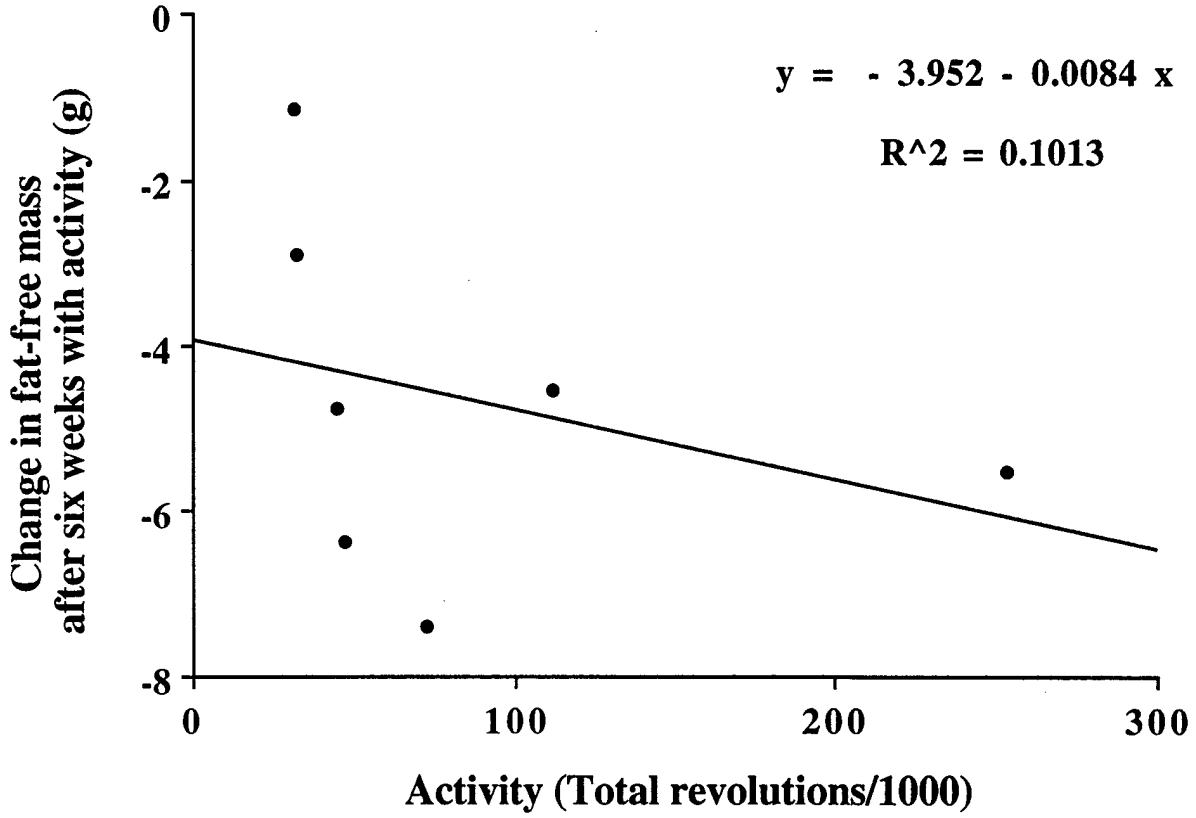


Figure 7.10. Relationship between the change in fat-free mass and activity level for voles on running wheels for six weeks. Slope of regression line was not statistically different from 0 ($p = .4867$). y = change in fat-free mass; x = activity (revolutions/1000)



Chapter 8

The effects of supplemental high-fat food on body composition of meadow voles (*Microtus pennsylvanicus*) in the wild

INTRODUCTION

Most small mammals (excluding hibernators) are quite lean (3-8 % body fat) in the field (Table 8.1). When brought from the field and held in the laboratory, they quickly increase body fat to 20 to 35 % (Batzli and Esseks 1992; Ferns and Adams 1974; Voltura 1996). In an 18 month study, we measured the body composition (using non-invasive EM-SCAN® procedures) of over 550 meadow voles (*Microtus pennsylvanicus*) fresh from the field. In that study, percent body fat ranged from 3 to 8 %. We also removed numerous voles from the field and maintained them in the laboratory under constant environmental conditions with lab chow (Lab Diet 5001, PMI Feeds) ad lib. Voles increased lipid mass and percent body fat rose to 20 to 25 % within four weeks, and then remained relatively constant. In addition, when the fat content of lab chow (5 %) was increased with added polyunsaturated fat (vegetable oil) to 25 %, and then fed to the same fattened voles, percent body fat further increased and was maintained at 25 to 30 % within three weeks. As a result, we know that meadow voles can deposit substantial amounts of body fat, but they never develop high fat levels in the field. We have found that the level of fat is related to diet.

Meadow voles are classified as generalist herbivores whose diet consists primarily of plant shoots and sedges. Diets composed of such vegetation are usually low in fat

(< 2 %) (National Research Council 1964, 1995) and relatively high in fiber (Keys and VanSoest 1970). Thus, meadow voles probably do not normally eat high fat diets in the field.

Prior food supplementation studies involving microtines in the field (Boutin 1990) have primarily emphasized effects on reproductive parameters such as litter size, breeding season length, and breeding intensity (Andrzejewski 1975; Cittadino et al. 1994; Cole and Batzli 1978; Desy and Thompson 1983; Ford and Pitelka 1984; Flowerdew 1973a; Taitt and Krebs 1981, 1983). But some studies have reported changes in population dynamics (immigration, emigration), body mass, and growth rate for voles when receiving supplemental food in the field (Cole and Batzli 1978; Desy and Thompson 1983; Flowerdew 1973b; Flowerdew and Gardner 1978; Hall et al. 1991; Krebs and Delong 1965; Taitt et al. 1981; Taitt and Krebs 1981, 1983; Saitoh 1989). However, the effects of supplemental food on body composition have not been reported.

We postulate that the lean body condition observed in the field is due to low quality forage, which is often relatively high in fiber and low in fat. If food quality is a cause of leanness in meadow voles in the field, could field supplementation of a high-fat diet increase fat content in resident animals? The objective of our study was to test whether meadow voles would increase body fat when given a high-fat food in a field setting. Since body composition changes observed within the laboratory could be due to stable environmental conditions, cage confinement, unnatural diets, or a combination thereof, we were interested in the effects of diet as a primary contributing factor in body

composition. In addition, we performed analyses of stomach contents to verify the overall fat content of natural ingesta in the wild.

MATERIALS AND METHODS

Fat content of natural ingesta

Meadow voles *(M. pennsylvanicus)* were trapped using "Museum Special" snap traps baited with a rolled oats and peanut butter mixture in a mixed-grass riparian field at the United States Air Force Academy, El Paso County, Colorado in August 1997. Traps were set approximately 1 h before dusk and checked within 1 h of sunrise. Since voles can spend up to 10 hours in a livetrap overnight during the summer months, consume the bait within the trap, and digest their natural ingesta, snap traps were selected to ensure that gut ingesta would not contain trap bait and should represent natural food. Captured voles were immediately taken to a laboratory at the United States Air Force Academy and dissected midventrally with the entire gastrointestinal tract (GI) (stomach to anus) removed. The stomach was then separated from the GI tract, the stomach contents removed, and stomach tissue discarded. Stomach contents were weighed to the nearest 0.0001 g (Mettler AJ100) and then dried at 60° C with a forced air convection oven until reaching constant mass (usually two days). Percent water of ingesta was determined as the difference in ingesta mass before and after drying. Stomach contents were ground in a coffee grinder and the entire contents were analyzed for lipid content using soxhlet extraction with petroleum ether by a contracted laboratory at the University of Western Ontario, London, Ontario, Canada.

Body Composition

This experiment was conducted during September/October 1997. Daylight photoperiod decreases during this period and may signal the onset of diminishing food conditions. The vegetation during this time period was still abundant but beginning senescence. The prevailing vegetation within these sites as described by Ripley (1994) includes smooth brome (*Bromus inermis*), cheatgrass (*Broma tectorum*), Kentucky bluegrass (*Poa pratensis*), thistle species (*Carduus nutans* and *Cirsium arvense*), snowberry (*Symphoricarpos occidentalis*). In addition, trapping sites included a mixed composition of narrow and broad-leaved cattails (*Typha angustigfolia* and *T. latifolia*), sedges (*Carex nabrascensis, Eleocharis palustris*, and *Schoenoplectus lacustris*) and rushes (*Juncus arcticus*). The surrounding tall and mixed grass communities are dominated by sandreed (*Calamovilfa longifolia*), big blue stem (*Andropogon gerardii*), blue gramma (*Bouteloua gracilis*), little blue stem (*Schizachyrium scoparium*) and needlegrass (*Stipa comata)*.

Two 20 m wide by 80 m long grids were established in a mixed-grass riparian field along a stream at the United States Air Force Academy, El Paso County, Colorado. Within each grid, feeding stations were placed every 5 m in width and every 20 m in length for a total of 25 stations per grid. These stations were constructed of a 30.5 cm long piece of black corrugated PVC tubing with a 12.7 cm diameter. For comparative purposes, one control grid was also established which did not receive supplemental food.

The control grid was 50 m wide and 200 m long and located one half mile from the experimental plots. All grids contained similar vegetation.

The period during which food was added to the experimental grids lasted for six weeks with trapping events scheduled for four consecutive days every three weeks. Supplemental food consisted of lab chow (Lab Diet 5001, PMI Feeds) with added fat (vegetable oil), resulting in a calculated dietary fat content of approximately 25 %. Vegetable oil was selected as the additive to maximize available unsaturated fat, the most prevalent lipid form in natural vegetation (National Research Council 1964, 1995). Lab chow was mixed with both water (4:1 by volume) and vegetable oil (4:2 by volume) to form a paste and then pressed into cakes with a quarter pound hamburger press and baked at 100° C for four hours. Then the cakes were dried at 50° C for 24 hours to remove as much water as possible. The lower temperatures for cooking and drying were selected to minimize fat vaporizing. Three separate batches of the high-fat diet were prepared. A proximate analysis (Nahm 1992) of each batch was performed by the Soil, Water, and Forage Analysis Lab at Colorado State. Results are presented in Table 8.2.

Food supplementation began on 1 September 1997. During the first three week period, each feeding station was supplemented with approximately 50 g of the high-fat food every three days. As a result, a total of 1250 g of added fat food was placed in each experimental grid every three days. Due to movement and disturbance of feeding stations during this period from an unknown source (perhaps deer, skunk, or raccoon), stations were anchored to each placement location with wire. We attempted to identify the cause of the station movement by searching for tracks during periods of wet weather and snow.

However, very few tracks were evident and those that were lacked identifiable characteristics. In addition, since food was missing from feeding stations 100 % of the time after three days, the resupply period was reduced to every two days for the remainder of the experiment. Thus, there was an overall increase in supplemental food of 33 % during the last three weeks of the experiment compared to the first three weeks. Three weeks was chosen as the trapping interval as prior lab experiments showed that voles will increase lipid mass to a plateau within two to three weeks when fed high-fat food.

Trapping sessions were conducted for four consecutive days beginning on 22 September 1997 and 15 October 1997. Sherman livetraps (22.9 x 7.6 x 8.9 cm) baited with Omalene (Poudre Supply and Feeds; Ft. Collins, CO) were set approximately 1 h before dusk and checked within 1 h of sunrise. Daylight trapping also occurred each day, with traps checked every two hours. In the control grid, two transect lines (10 m separation) consisting of 40 traps each were placed in vole runways with approximately 5 m between traps. As a result, a total of 320 trap nights were made each 4 day trap session. In each supplemental grid, one trap was placed facing out at each end of the PVC tube within each feeding station resulting in 50 trap nights per grid and a cumulative total of 200 trap nights per four day session.

Feeding station visitation

The extent of feeding station visitation was investigated during the final four day trapping period. At each station, traps were placed at each end of the PVC tube facing out. Food was dusted with fluorescent powder (orange, #R3-GR1101, Radiant Color,

Inc., Richmond, CA. in grid 1; green, #R3-OG1103, Radiant Color, Inc., Richmond, CA. in grid 2) and placed in the center of each station. The inside of the PVC tubing was also dusted with powder, with precautions taken to ensure no powder was present on the outside of the PVC tube or on the ground. Powdered fluorescent pigments have low toxicity, are inexpensive, and transfer readily from one surface to another upon contact. Application onto the fur of small mammals along with ingestion of dusted foodstuffs present little risk to the health of study animals (Stapp et al. 1994). Fluorescent powder is ideal for documenting small mammal movements in the field, (Lemen and Freeman 1985, 1986; Jike et al. 1988; Mullican 1988; Longland and Clements 1995), to assess the use of microhabitat (Barnum et al. 1992; Goodyear 1989; Graves et al. 1988; McShae and Gills 1992), foraging ranges (Hovland and Andreassen 1995), and social interactions (Kaufman 1989; Getz and Hoffman 1986; Getz et al. 1992, 1993). During daylight hours, ten stations in each grid also received an additional glue-board rodent trap placed at one end of the PVC tube. Positive feeding station visitation was determined if: 1) food was removed and missing from the feeding station; 2) food was moved to one edge of feeding station; and/or 3) if visible bite marks were present in the remaining food. Since insect populations are low during this time period, and no other small mammal but meadow voles had been captured in a previous 18 month trapping study, it was assumed that bite marks were the result of meadow vole activity.

Captured voles were immediately given an apple slice and piece of lab chow (Lab Diet 5001, PMI Feeds) in order for them to rehydrate and provide gut-fill following trap retention. Then, voles were held within capture traps and transported to a laboratory at

the United States Air Force Academy within one hour of capture to measure body composition. Body composition was measured using a non-invasive EM-SCAN ® procedure as in Voltura and Wunder (1998).

So that no field animal was sampled twice during a trapping session, voles were marked with pink nail polish on the tail, the bottom of all feet and the top of the head while under anesthesia for EM-SCAN® measures. This ensured that polish was not licked off prior to drying. Once body composition measures were completed, each vole was returned to it's capture location for release. During all trapping sessions, if voles were recaptured within the four day period, they were immediately released and not remeasured for body composition. In addition, each vole and feces within capture traps were placed under ultraviolet light to determine if fluorescent dye was present, which would indicate a positive feeding site visitation.

Data collected on each vole included measures of total body mass (Ohaus E400D) combined with estimates of fat-free mass and lipid mass using EM-SCAN®. In addition, percent lipid mass and percent fat-free mass were calculated by dividing lipid mass and fat-free mass by total body mass.

Statistics

Comparisons of body composition parameters (total body mass, lipid mass, fat-free mass, percent lipid mass, percent fat-free mass) between all grids were made with ANOVA (SAS, proc glm) (SAS Institute, Inc. 1989) using least squared difference criteria. For all comparisons, statistical significance was set at $p < 0.05$.

RESULTS

Fat in natural vegetation

Lipid analyses of stomach contents from eight voles showed a dry matter lipid mass of 0.04 ± 0.01 g. This equates to a wet stomach content of 4.14 ± 0.83 % total fat. The range of data for percent fat was 1.56 to 8.39 %. No analysis of ingesta species composition was performed. Percent water of ingesta was 74.17 ± 5.41 %, which is consistent with the high water content of the primary vegetation in which meadow voles feed.

Body composition

There was no significant difference in any body composition parameter between the experimental grids and the control area for the first three week period (Figure 8.1; Table 8.3). However, during the second three week period, we found significant differences between treatments for lipid mass ($F_{2,39} = 5.86$; $p = 0.0059$), percent lipid mass ($F_{2,39} = 6.06$; $p = 0.0051$), and percent fat-free mass ($F_{2,39} = 6.06$; $p = 0.0051$), but not for total body mass or fat-free mass. (Figure 8.2; Table 8.3). Multiple comparisons of means between grids resulted in significant differences in lipid mass ($p = 0.0015$), percent lipid mass ($p = 0.0012$) and percent fat-free mass ($p = 0.0012$) between experimental grid 1 and control. Grid 1 had a mean lipid mass of 3.78 ± 0.62 g as compared to 1.75 ± 0.23 g in the control. In addition, percent body fat in grid 1 was 8.74 ± 1.27 % as compared to 4.44 ± 0.50 % in the control area (Figure 8.3). In contrast, we found no significant differences between grid 2 and the control area for any body composition parameter,

although variation in lipid mass ($p = 0.0643$), percent lipid mass ($p = 0.0632$) and percent fat-free mass ($p = 0.0632$) was nearly significant (Table 8.3). There were no significant differences in body mass, lipid mass, and fat-free mass between grids receiving supplemental food during either trapping period (Figure 8.1, 8.2; Table 8.3).

Closer investigation of lipid mass for control area captures during both trapping periods reveals that only 2 out of 63 voles had a body fat level greater than 10 %. Body fat levels of these voles were similar to those found in an 18 month field study we completed, in which less than 10 % of 568 captures exceeded 10 % body fat. The percent body fat levels of these voles were also consistent with the 3 to 6 % body fat range found in the 18 month study.

In the initial three week supplementation period, only one vole out of 20 captures exceeded 10 % fat. In contrast, the second trapping period resulted in 6 of the 12 captures in grid 1 with body fat levels greater than 10 %. During the same period, two voles out of 12 captures were also greater than 10 % body fat in grid 2. By pooling the data from both supplemental grids, 33 % of voles exceeded 10 % body fat during this period. These results are inconsistent with body composition previously observed in these locations and represent a much higher percentage of individuals with atypical body composition (high body fat). We interpret these results to be consistent with the hypothesis that an added high-fat diet can increase body fat of voles living in the field.

Feeding site visitation

To determine the extent of supplemental feeding station visitation, daily checks at each of the 50 total feeding stations were made while checking livetraps, resulting in a total of 200 observations. A total of 171 of these observations (85.5 %) were classified as positive feeding station visitation. Food was missing from the feeding stations a total of 74 times. In addition, food was moved to the edge of the feeding station or visible bite marks were present in the food a total of 97 times (Figure 8.4). Both supplemental grids demonstrated consistent results. Grid 1 had a positive visitation rate of 95 %, while grid 2 had 86 % positive visitation. In contrast, glue traps placed at the entrance of 10 traps in each grid resulted in no captures of voles or insects. Whether the lack of success was due to lack of visitation or capture flaws inherent in such a trapping device is unknown.

The presence of fluorescent dye on captured voles or on the feces in capture traps is also noteworthy. During the second trapping session, six of the twelve voles captured in grid 1 had visible dye on their fur. Of these subjects, four were voles which had a body fat of over 10 %. In addition, one vole in zone 2 had dye presence, but this individual did not have high body fat.

DISCUSSION

Although voles on the supplemental grids had a higher mean body mass than voles on the control grid, these masses were not significantly different (Table 6.3). These results were inconsistent with the observations of Andrzejewski (1975), Boutin (1990), Desy and Thompson (1983), Flowerdew (1972), Flowerdew and Gardner (1978), Taitt

(1981) and Taitt and Krebs (1981) in which body mass increased with supplemental food. Although not specifically addressed in those studies, the observed body mass changes might also incorporate changes in body composition similar to our study. However, no investigation of body composition dynamics, besides body mass, has been previously reported following food supplementation.

The presence of a positive correlation between increased lipid levels and supplemental food does not prove that there is a causal relationship. However, the reasons for thinking that the relationship is causal are strong. The high percentage of voles that we found with atypical body fat levels within the supplemental grids suggests the effect of dietary quality on body lipid. In the wild, meadow voles with greater than 10 % body fat are atypical. Monthly variation between 3 to 6 % body fat is more commonplace (Table 8.1), with even lower body fat levels present during gestation and lactation in females (Voltura 1997). With 50 % of the captures in one grid, or 33 % of the voles in both supplemental grids having an atypical body composition (> 10 % body fat), we believe that the added high-fat food may have contributed to this result.

The fattening observed on the experimental grids is also noteworthy because it links observations from the lab to the field. When brought into the lab from the field and fed lab chow *ad lib.*, voles increase body fat from 5 to 25 % within four weeks. This lab fattening has been attributed to constant environmental conditions, unnatural diets, lack of activity due to cage confinement, or combinations thereof (Batzli and Esseks 1992; Donald et al. 1980; Ferns and Adams 1974; Voltura 1996). Although the fat levels we observed in the supplemental grids (9 %) were not as high as those observed in the lab,

they were significantly higher than voles from the control area and were very atypical for voles from the field. The reasons that the body fat level of voles in the experimental grids did not reach the levels observed in the lab may be due to habitat dynamics and its effect on body composition. In addition, voles may only receive part of their daily food intake from the supplemental food. In the lab, the entire intake consists of the high-fat food. In the field, variations in ambient temperature variation also affect thermogenic demands (Wunder 1984). In addition, greater activity levels may be present and ever-changing food abundance and quality may also influence body composition differently than in a lab.

The results of the lipid analyses of stomach ingesta were slightly higher than we expected. In contrast to the fat content of 4 % in our study, a fat content of around 2 to 3 % is most common in non-reproductive grass shoots and sedges (NRC 1964, 1995). However fat content can vary dependent upon the stage of growth in plants. Voles may be selecting new shoot growth which may be higher in fat content. Nevertheless, this confirms that meadow voles consume a diet which is relatively low in available fat. The higher fat content of the ingesta may be the result of voles targeting vegetative components with higher fat (seeds), animal matter, or tubers. In addition, voles which we collected may have consumed trap bait (peanut butter and oat mixture) from other traps which were tripped and unsuccessful in capturing animals. Although less likely, voles may also have consumed trap bait from successful traps before the snap mechanism was tripped.

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Table 8.1. Percent body fat of wild, non-hibernating small mammals.

Species	Fat Content	Reference
Meadow vole (*Microtus pennsylvanicus*)	3 - 7	1
	3 - 8	10
Pine vole (*Microtus pinetorum*)	5 - 7	6
Prairie vole (*Microtus ochrogaster*)	4 - 5	7
	3 - 5	9
	4 - 6	2
Red-backed voles (*Clethrionomys gapperi*)	3 - 5	1
Brown lemming (*Lemmus sibiricus*)	3 - 5	3
Deer mouse (*Peromyscus maniculatus*)	3 - 5	8
	5 - 7	4, 7
White-footed mouse (*Peromyscus leucopus*)	5 - 8	5
Harvest Mouse (*Reithrodontomys megalotis*)	3 - 5	8
	6 - 7	4
Grasshopper mouse (*Onychomys leucogaster*)	4 - 6	8

1. Anderson and Rauch 1984
2. Baker 1971
3. Batzli and Esseks 1992
4. Fleharty et al. 1973
5. Lynch 1973
6. Lochmiller et al. 1983
7. Morton and Lewis 1980
8. Schreiber and Johnson 1975
9. Voltura 1997
10. This study

Table 8.2. Proximate analysis of three separate batches of high-fat diet (lab chow + vegetable oil). One cake per batch was analyzed. Column values represent composition as received (A). Cakes were further dried to constant mass by the analysis lab and reported on a dry matter basis (B).

	Batch #1		Batch #2		Batch #3	
	A	B	A	B	A	B
Percent (%)						
Moisture	9.97	0.00	22.30	0.00	20.77	0.00
Dry Matter	90.03	100.00	77.70	100.00	79.23	100.00
Crude Protein	19.21	21.34	15.62	20.02	16.35	20.69
ADF	9.83	10.92	8.10	10.38	8.46	10.71
Nitrogen	3.08	3.42	2.50	3.20	2.62	3.32
Phosphorus	0.51	0.57	0.36	0.46	0.43	0.55
Potassium	1.02	1.13	0.80	1.03	0.92	1.17
Calcium	0.77	0.85	0.55	0.71	0.66	0.84
Magnesium	0.18	0.20	0.12	0.16	0.15	0.19
Sodium	3.01	3.34	1.89	2.42	2.46	3.14
Mg/kg (ppm)						
Iron	209.1	232.2	143.4	183.8	185.7	235.1
Manganese	55.03	61.14	41.18	52.80	49.96	63.24
Zinc	70.00	77.78	47.48	60.87	59.03	74.72
Copper	14.35	15.94	10.29	13.19	11.38	14.40
Boron	10.09	11.21	5.11	6.55	6.22	7.87
Molybdenum	1.71	1.90	1.65	2.11	1.32	1.67

Table 8.3. Comparison of body composition parameters between the two supplemental feeding grids and control grid. Values are mean plus/minus one standard error (in parentheses). ANOVA F-value is for three way comparison. Significant differences between grids ($p < 0.05$) annotated with different letters.

Time & Condition	F Value ANOVA	Grid 1	Grid 2	Control Grid
Initial 3 week period	df (2,60)	n = 10	n = 9	n = 44
Mass (g)	.7657	40.15 (4.19)^{A}	38.49 (4.65)^{A}	41.58 (1.72)^{A}
Lipid mass (g)	.1593	1.47 (0.29)^{A}	1.78 (0.45)^{A}	2.23 (0.18)^{A}
Fat-free mass (g)	.8266	38.68 (4.18)^{A}	36.71 (4.43)^{A}	39.35 (1.66)^{A}
Percent Lipid mass (%)	.2071	3.94 (0.66)^{A}	4.44 (1.08)^{A}	5.50 (0.41)^{A}
Percent Fat-free mass (%)	.2071	96.06 (0.66)^{A}	95.56 (1.08)^{A}	94.50 (0.41)^{A}
Final 3 week period	df (2,39)	n = 12	n = 12	n = 19
Mass (g)	.5844	44.21 (3.82)^{A}	40.88 (2.69)^{A}	39.91 (2.60)^{A}
Lipid mass (g)	.0059	3.78 (0.62)^{B}	2.54 (0.48)^{AB}	1.75 (0.23)^{A}
Fat-free mass (g)	.8352	40.43 (3.65)^{A}	38.34 (2.53)^{A}	38.15 (2.50)^{A}
Percent Lipid mass (%)	.0051	8.74 (1.27)^{B}	6.15 (1.03)^{AB}	4.44 (0.50)^{A}
Percent Fat-free mass (%)	.0051	91.26 (1.27)^{B}	93.85 (1.03)^{AB}	95.56 (0.50)^{A}

Figure 8.1. Comparison of body composition parameters between grids during the 1st trapping session. Values are mean plus/minus one standard error (error bars). Different letters indicate significant differences between grids ($p < .05$).

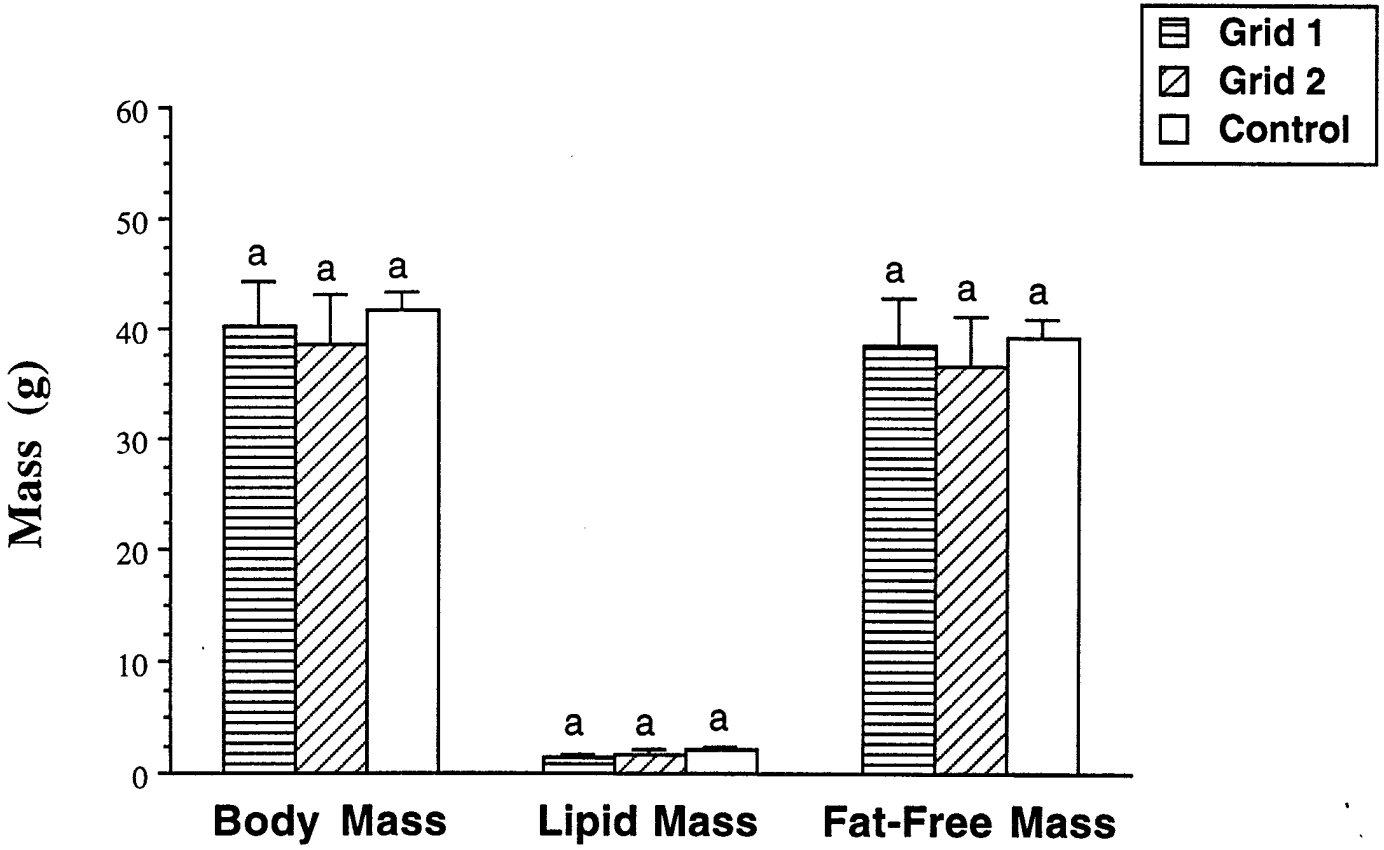


Figure 8.2. Comparison of body composition parameters between grids during the 2nd trapping session. Values are mean plus/minus one standard error (error bars). Different letters indicate significant differences between grids ($p < .05$).

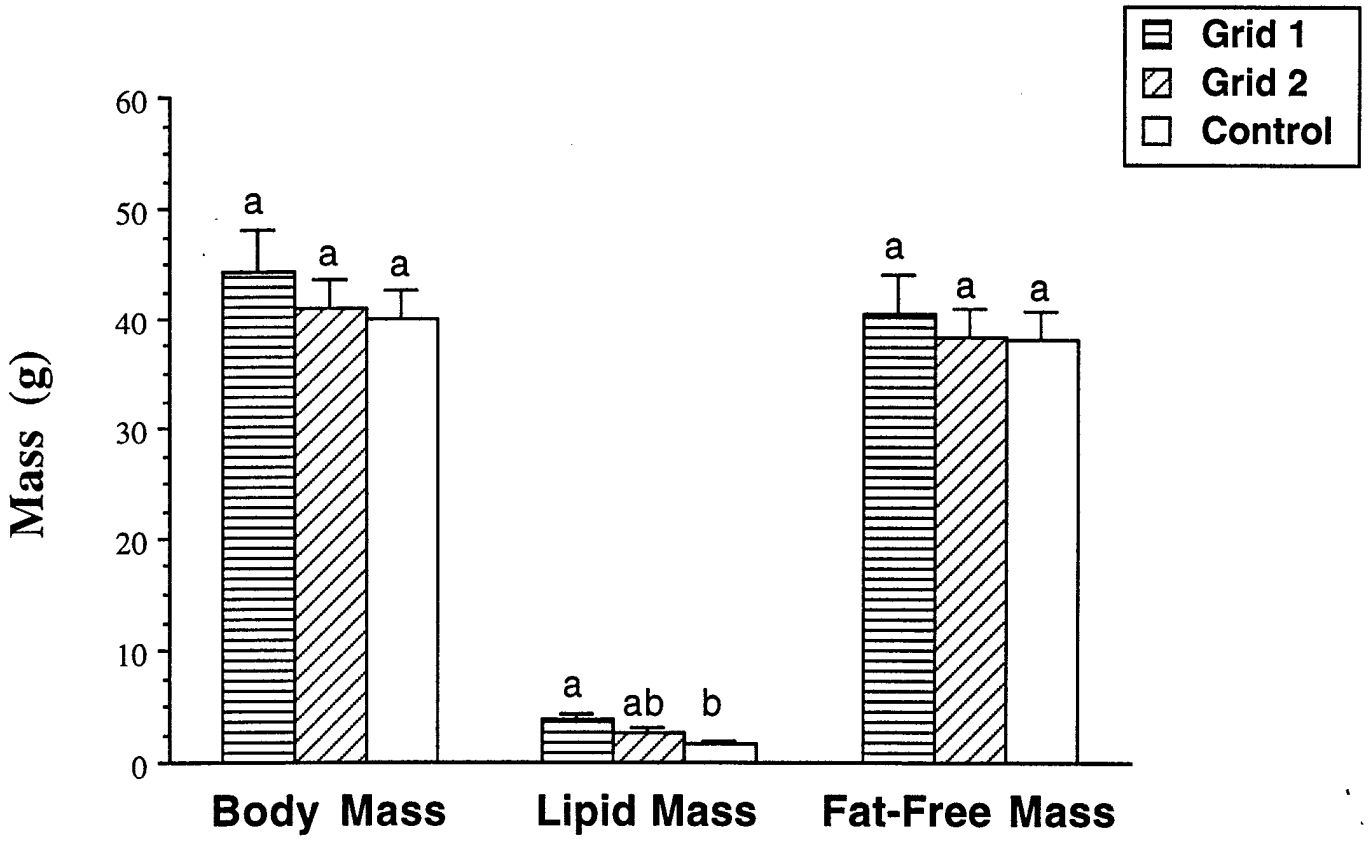


Figure 8.3. Comparison of percent lipid mass between grids during both trapping sessions. Values are mean plus/minus one standard error (error bars). Different letters indicate significant differences between grids ($p < .05$).

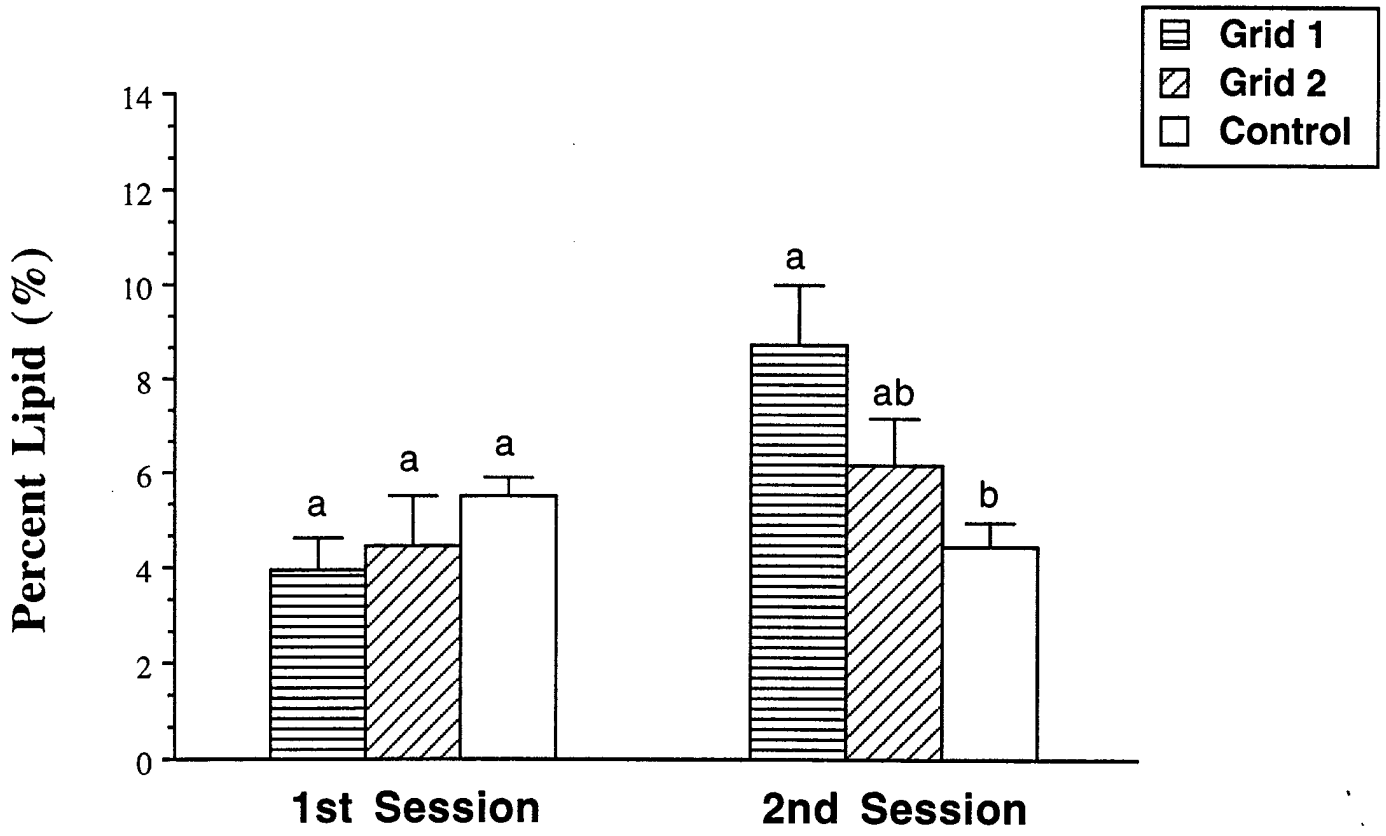
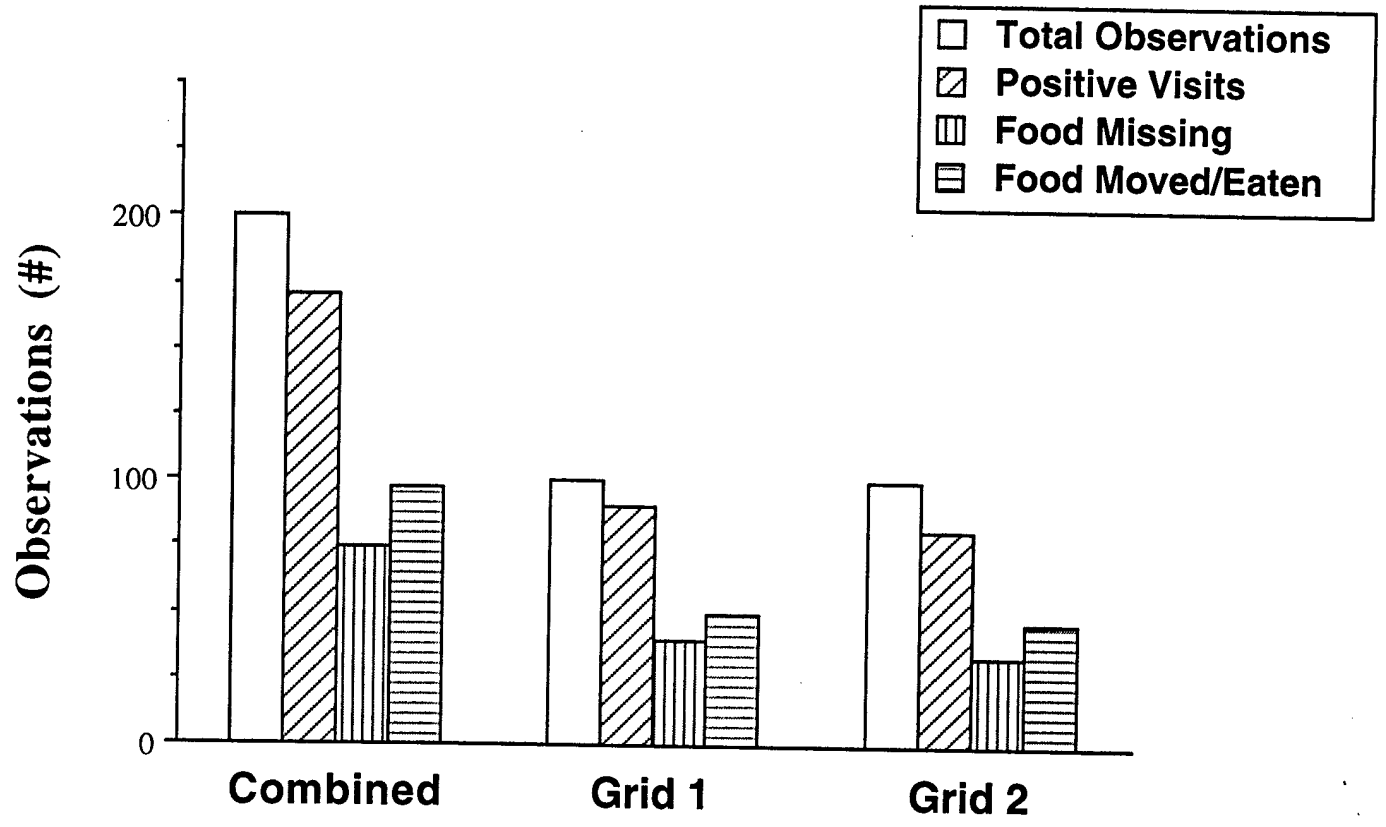


Figure 8.4. Summary of feeding station visitation between grids during 2nd trapping session. Values are sum of observations over a four day period. Combined are sum of grid 1 and grid 2.



Chapter 9

Research Summary

From the results presented in the previous chapters, we were able to gain additional insights into body composition dynamics in meadow voles, as well as confirm the application of EM-SCAN® for noninvasive body composition studies. In general, meadow voles in the wild are relatively lean year-round, maintaining from 2-3 g of lipid mass regardless of season. The proximate cause for this lean body condition may be due to diet quality. We showed that voles will select a high fat diet when given a choice of foods varying only in dietary fat. One would expect similar behavior in the field with voles selecting high fat food. However, their lean body composition in the wild suggests that foods high in fat are not readily available or such foods are not selected due to the high abundance of readily available vegetation with lower fat content. Indeed, if voles are selecting vegetation or plant parts with high fat, then one would expect voles in the field to have higher lipid mass and percent body fat. In addition, we reported that voles deposit large amounts of lipid mass within a few weeks when held under laboratory conditions and fed laboratory diets. Regardless of diet, voles did not continue to increase body mass. Rather, they showed a plateau in body mass for each diet, which suggests an endogenous regulation of body composition corresponding to dietary quality and abundance. We also believe that activity has little effect on the lean condition of voles in the field. In the lab, voles ran an average of 1.07 ± 0.44 kilometers per day over six weeks and still gained an average of 12 g of lipid mass and increased percent body fat to 30.5 % (levels which were actually higher than control voles without running wheels). As a result, we conclude that

in the laboratory, running has little effect on fattening and may even enhance lipid deposition.

The importance of body fat in meadow voles remains unclear, although we believe that body fat plays a relatively minor role in overwinter survival. To ensure survival during winter, meadow voles rely primarily on energy sparing where adult animals decrease body mass and subadults reduce growth rates. This strategy reduces energy requirements by decreasing the total fat-free mass, a high energy requiring tissue, without the need to increase lipid mass. During winter, voles also increase percent body fat from 4 to 7 % by maintaining lipid levels and decreasing overall body mass. However, the biological relevance of this small seasonal change remains unknown.

In studies of body composition, we highly recommend the EM-SCAN® device as an accurate and reliable method. Able to estimate lipid mass levels to within 1 to 2 g, EM-SCAN® provides a means to investigate body composition with a high degree of confidence. Although this level of estimation accuracy may result in a 100 % error in lipid mass levels in very lean animals, it still can provide valuable information concerning body composition. Whether a vole is estimated to have 2 or 3 g of lipid mass, a 50 g animal would still be considered very lean, regardless of such high estimate error. In situations where animals have much higher body fat, the relative error in estimates of lipid mass is greatly improved. The use of EM-SCAN® as a tool where very precise measures of body composition are necessary may be inappropriate. However, its ability to provide repeatable, noninvasive information cannot be overlooked. In field applications with EM-SCAN®, we suggest that captured animals are allowed to recover from potential

dehydration or reduced gutfill during trap retention before body composition measures are made. Within 2 to 3 hours, voles given apple will regain lost body water and achieve gutfill levels sufficient to minimize the effects of livetrap retention on body composition. To further reduce changes in body mass and body composition in livetrap methods, we also suggest using apple as a trap bait.

We have shown that when wild meadow voles are removed from the field and held in the lab, they dramatically change body composition. Within several weeks, voles increase body mass and lipid mass to levels atypical of field animals. Although we did not investigate whether these changes in body composition actually affect physiological performance in individuals, we feel that research in such areas is warranted. In addition, researchers should question whether laboratory results with lab-reared or field-captured animals (which are kept in the lab) are relevant to animals in the field. Lastly, whether body composition change can be reduced or negated when wild animals are held under laboratory conditions should be investigated.