

Variation in body composition of female big brown bats (*Eptesicus fuscus*) during lactation

Wendy R. Hood · Olav T. Oftedal · Thomas H. Kunz

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Abstract Most small mammals support the nutritional requirements of milk production by increasing food intake. However, when nutrient intake is low, maternal body reserves may be mobilized to maintain adequate milk output. We examined patterns of body composition, including dry matter, fat, protein, and mineral content in big brown bats, *Eptesicus fuscus*, during lactation. Concentrations of fat and phosphorus were markedly lower in lactating mothers during week three of lactation than during the first two weeks, but these constituents rebounded to previous levels in the fourth and fifth week. Rapid recovery from fat depletion suggests that females are able to adjust to changes in demands for energy. The decrease in phosphorus during mid-lactation suggests bone demineralization, but an interspecific comparison of adult concentrations of minerals prevalent in bone suggests that mineral concentrations may never reach critically low levels in reproductively active females.

Keywords Body composition · Total body fat · Total body calcium · Lactation · *Eptesicus fuscus*

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W. R. Hood · T. H. Kunz
Department of Biology, Boston University, Boston, MA
02215, USA

W. R. Hood (✉)
Department of Biology, Coastal Carolina University,
PO Box 261954, Conway, SC 29528, USA
e-mail: wrhood@coastal.edu

O. T. Oftedal
Department of Conservation Biology, National Zoological
Park, Smithsonian Institution, Washington, DC 20008, USA

Introduction

During lactation, mothers are faced with the challenge of acquiring sufficient energy and nutrients to meet the demands of their growing offspring, while maintaining their own body condition. This tradeoff may be especially tenuous in small volant mammals, when 20–40% of mother's daily energy expenditure may be devoted to milk production and the amount of nutrients that can be stored prior to lactation are restricted by small body size and the constraints on body mass associated with flight (Racey and Speakman 1987; Oftedal 2000). The immediate nutritional requirements of most animals are met largely by consuming a nutrient-rich or varied diet. When food intake is insufficient, as is expected during the peak energy demand of lactation, some mammals will lower their own metabolic energy requirements by reducing their activity and in some cases reducing their body temperature (Audet and Fenton 1988; Geiser 1996; Grinevitch et al. 1995; Hamilton and Barclay 1994). Nutrients stored in the body prior to lactation also may be mobilized (Dominey 1984; Racey and Speakman 1987).

Because nutrient storage is limited and excessive depletion of the body's nutrient reserves may ultimately impair reproductive performance (Clutton-Brock et al. 1982), compromise an animal's health (McDowell 1992), and perhaps increase its vulnerability to predation (Sweitzer and Berger 1992), small mammals are likely to deplete body stores as a last resort when demands cannot be met by intake. Some fluctuations in body composition may be associated with normal homeorhetic changes that occur during lactation, such as bone demineralization independent of dietary intake (Prentice 2000; Kovacs 2005). How-

ever, substantial changes in body composition in lactating small mammals are most likely to occur during peak lactation, reflecting discrepancies between dietary intake and metabolic requirements.

The risks associated with changes in body composition may be especially pronounced in bats, because changes in the body mass of bats are known to influence flight and foraging ability (Webb et al. 1992). Modifications to wing structure, such as decalcification of wing bones, may reduce a bat's ability to withstand torques associated with powered flight (Papadimitriou et al. 1996; Swartz et al. 1992). Several investigators have suggested that reproductive patterns exhibited by insectivorous bats may be influenced by the relatively low calcium content of their diet (Barclay 1994, 1995; Kwencinski et al. 1987; Studier et al. 1991, 1994a). Young bats do not reach nutritional independence until they reach approximately 70% of adult mass, nearly twice the relative mass of other terrestrial mammals that are typically weaned at 40% of adult mass (Barclay 1994, 1995). This extended dependence allows for full development of wings and calcification of forelimbs and digits required for flight (Papadimitriou et al. 1996; Swartz et al. 1992). Because mothers supply their young with their only source of calcium for much of their postnatal development (Spray and Widdowson 1950; Widdowson and Dickerson 1964; Studier and Kunz 1995), Barclay (1994) postulated that insectivorous bats may be unable to channel enough calcium into their milk to support more than one or two young to weaning.

In this study, we examine patterns of total body macronutrient and mineral composition in lactating female big brown bats (*Eptesicus fuscus*). We predicted that changes in maternal body composition could reveal discrepancies between maternal dietary intake and nutritional demands of lactation, both of which are likely to vary over the course of lactation. Since bone demineralization appears to be typical of lactation (Prentice 2000; Kovacs 2005) and calcium intake is likely to be relatively low on an insectivorous diet (Studier and Sevcik 1992), we predicted that lactating mothers would mobilize calcium and phosphorus from bone hydroxyapatite (Goodman 1994), resulting in concurrent loss of body calcium and phosphorus.

Materials and methods

During June and July 1999, we collected lactating female big brown bats and their twin offspring by hand or with hand nets from barn-roosting maternity colonies in Hollis and Milford, New Hampshire, USA (42°74' N

71°59' W, 42°83' N 71°68' W, respectively). Each week, we collected four to seven mothers between 0600 and 0900 hours and brought the bats back to Boston University where we placed them in an incubator maintained at a temperature within the bat's thermoneutral zone (30°C, Stack 1985). To assure that all individuals were post-absorptive, we held the bats overnight. At ~ 2100 hours, we gave the bats water ad lib, via a syringe to prevent dehydration. The following morning, we over-anesthetized the bats with chloroform. The body composition of pups is described elsewhere (Hood 2001). We measured the body mass and forearm lengths of the bats and examined the relative wear of their canines post-mortem. Canine teeth of big brown bats wear over time and thus can be used as a measure of relative age (Christian 1956; Holroyd 1993). We scored the canines on a scale of one to three; sharp canines typical of young animals were assigned a score of one whereas extremely worn canines, typical of old animals, were assigned a score of three. A detailed description of this scale can be found in Hood et al. (2002).

We opened the abdominal cavity of each bat by incision and removed the combined large and small intestines to evaluate gut fill and changes in intestinal mass described elsewhere (Hood 2001). We severed the small and large intestines from their attached mesenteries within a few millimeters of the intestinal wall, leaving a small amount of mesentery associated with the intestine to prevent damage during removal. To minimize water loss from the opened carcass, we immediately sealed carcasses in Whil-pacTM plastic bags, as soon as the intestines had been removed, after an average of 2 min from initial incision (range 1–5 min). We floated the intestines in saline to minimize evaporative water loss, sectioned the intestine to allow removal of contents, blotted it dry to remove saline, and then reweighed and sealed the tissue into an air tight bag. For most individuals, the intestine was empty but a few individuals had intestinal worms and/or one or two fecal pellets in the distal colon. We estimate the mass these items in all cases to be under 0.1 g; the mass lost by the carcass and intestine during dissection averaged 0.23 g (hereafter ML). We found no correlation between dissection time and ML ($R^2 = 0.06$, $P = 0.27$). Given that the intestines were largely empty and that ML may have been altered by floating in saline and blotting, we considered the mass of the intact animals prior to dissection to represent empty body mass (i.e., mass of the animal devoid of gut contents). We froze both the open carcass and the intestines at –80°C until further analysis.

We adopted a molecular model for the analysis of body composition, whereof the body was divided into

four compartments, water, fat, protein, and mineral (Malina et al. 2004). This model assumes that carbohydrate storage as glycogen is minimal. We homogenized thawed carcasses in a Corning commercial blender. We determined the dry matter content of the animals by mass change during drying to a constant mass at 60°C. We further homogenized dry samples in a Wiley Mill with a 2 mm mesh sieve. We performed all subsequent analyses in duplicate. Targeting neutral storage lipids, we determined fat composition based on the mass of fat extracted in 500 ml of petroleum ether in a Soxhlet apparatus for 15 h (0.516 ± 0.021 g samples; method adapted from Osborne and Voogt 1978). We retained the fat-free residues for nitrogen and mineral analysis. We determined nitrogen content of 8.48 ± 1.80 mg samples by CHN analysis (Perkin Elmer PE2400 Series II CHNS/O Elemental Analyzer, Shelton, CT) and converted total nitrogen to crude protein by using the conversion factor of 6.25 (Robbins 1993). For mineral analyses, we digested 98.4 ± 10.8 mg sample with a microwave accelerated reaction system (MARS5, CEM Corp., Mathews, NC) in 10 ml concentrated nitric acid, ramped to 220°C over 15 min and held at 220°C for an additional 15 min. We diluted these digests to approximately 20 g with distilled deionized water and determined the mineral content (other than phosphorus) of these solutions with atomic absorption spectrophotometry (Smith-Hieftje 12, Thermo-Jarrell Ash, Franklin, MA). We added modifiers to sample aliquots to reduce interferences, including lanthanum chloride to a final concentration of 4,000 ppm lanthanum for calcium analysis and 1,000 ppm lanthanum for magnesium analysis, potassium chloride to a final concentration of 2,000 ppm potassium for sodium analysis, and cesium chloride to a final concentration of 1,000 ppm cesium for potassium analysis. We measured magnesium, sodium, and potassium using an air-acetylene flame at wavelengths of 202.5 nm, 589.0 nm, and 766.5 nm, respectively and calcium using a nitrous oxide-acetylene flame at a wavelength of 422.7 nm. We analyzed phosphorous using the AOAC-modified Gomori molybdovanadate method (Horwitz 1980). We analyzed National Institutes of Standards and Technology non-fat dry milk powder (reference material # 1549, U.S. Department of Commerce, Gaithersburg, MD) to determine the accuracy and precision associated with mineral analyses. The measured concentrations of minerals in the standard were Ca = 12.13 ± 0.32 mg/g, P = 10.8 ± 0.2 mg/g, Mg = 1.20 ± 0.02 mg/g, K = 15.58 ± 0.88 mg/g, and Na = 4.61 ± 0.05 mg/g ($n = 12$, except Na where

$n = 6$). Measured concentrations of phosphorous, magnesium, and sodium were not different from the declared values, but calcium and potassium were slightly lower, with recoveries of 93.3 and 92.3%, respectively. We pooled residual gut samples and analyzed them using the same methods as carcass samples. Based on the mass of the gut removed and the nutrient concentration of the residual gut, we adjusted total body composition to include the estimated composition of the gut.

We assigned stage of lactation by date of capture. Because a few mothers were not captured with their young attached to the nipple, we could not confirm the relationship between independent young and their mothers. Thus, we could not use morphometric measurements of pups to estimate day of lactation. We designated the week that pups were first observed in each colony as week 1 of lactation. During week 1, all pups were hairless, eyes of most pups were still closed (an indicator that pups were less than two days old, Kunz 1974), and several females were still pregnant. We assigned lactating females collected 7 days later to week 2 and subsequent collections to weeks 3, 4, etc. Because 89% of births occur within a 7 day period (Hood 2001), we are confident that most individuals were correctly classified. In the event that we classified animals incorrectly, the error would only be one week.

We completed statistical analyses using SAS (SAS Institute, Inc., Cary, NC). For all analyses, we arcsine transformed proportional data. We expressed nutrients relative to total body content using units that would reveal functional trends rather than the confounding effects of change in other constituents. For example, during postnatal development, the proportion of total body water decreases primarily due to decreasing extracellular fluid (Spray and Widdowson 1950; Widdowson and Dickerson 1964). Expressing nutrients on a wet mass basis would lead to an apparent increase in nutrient concentration due to the decline in body water. Change in fat deposition can also alter whole body nutrient concentrations independent of relative changes within the fat-free body (Spray and Widdowson 1950; Widdowson and Dickerson 1964). To separate the effects of water and fat, we expressed body water content relative to fat-free mass (FFM) and fat relative to dry mass (DM). We expressed protein relative to fat-free dry mass (FFDM), as were calcium, magnesium, and phosphorus, since the storage of these minerals is not associated with water or fat compartments. Although associated with electrolytic function, we also presented sodium and potassium relative to the fat-free dry mass of the animals for consistency with other minerals and to facilitate comparison to other studies.

We used general linear models to compare variation in body mass with week of lactation, colony, and relative maternal age. We compared maternal body composition to week of lactation, colony, and relative maternal age using multivariate analysis of variance (MANOVA, proc GLM) with a Scheffe adjustment of multiple comparisons (SAS Institute Inc. 1990). Because they are not considered independent, we included all body constituents in the omnibus test. Colony and relative maternal age were not significant variables (see results) and thus we dropped these variables from the final model. Finally, we compared Ca:P with week of lactation using general linear models. For comparison, we report data on body composition from others studies in Tables 1–5. The results of Spray and Widdowson (1950; Tables 1, 2) were originally presented as bivariate graphs and thus exact values were not available. We estimated values by digitizing these graphs using the computer program DigiMATIC (FEB software, Chesterfield, VA). Units were converted as necessary for comparison to the present study. These tables include representative studies and thus do not include an exhaustive list of published research on chemical analysis of body composition of these or other species.

Results

Our analyses of body constituents accounted for $96.5 \pm 0.8\%$ of the body mass of each bat (Tables 1, 2). The three major age classes of adult female bats were nearly equally represented by our data (nine young, seven middle aged, and nine old), although there was a slight skew in colony representation with 15 bats collected in Milford and 10 collected in Hollis. Body mass did not change with the week of lactation or differ with colony or relative maternal age ($F = 1.35$, $df = 9, 15$, $P > 0.29$, Fig. 1a). Variation in body composition between colonies and with relative maternal age was not significant (colony: Wilk's $\Lambda = 0.633$, $F = 0.72$, $df = 8, 10$, $P > 0.670$; maternal age: Wilk's $\Lambda = 0.223$, $F = 1.40$, $df = 16, 20$, $P > 0.238$). Thus, colony and maternal age were excluded from the model.

Body water, as a percent of total fat free mass, remained relatively constant, averaging to 69.3% (Table 1, $F = 2.09$, $df = 4, 20$, $P > 0.12$). Similarly, the FFDM concentrations of protein (Table 1), magnesium, potassium, and sodium content (Table 2) of the body did not vary by week of lactation ($P > 0.05$). During the third week of lactation, percent fat (DM, Fig. 1b) and phosphorous (FFDM, Fig. 2b) decreased; calcium (FFDM) also appeared to decline during this

week (Fig. 3a) but this change was not significant (MANCOVA, overall: Wilk's $\Lambda = 0.012$, $F = 3.57$, $df = 32, 49$, $P < 0.001$; fat: $F = 15.5$, $df = 4, 20$, $P < 0.001$, Figure 1b; phosphorus: $F = 8.92$, $df = 4, 20$, $P < 0.003$, Figure 2b; calcium $F = 2.23$, $df = 4, 20$, $P > 0.10$; Figure 2a). The ratio of calcium to phosphorus differed during lactation between weeks two and five, with a greater proportion of calcium observed during week five ($F = 3.53$, $df = 4, 20$, $P < 0.0225$, Fig. 2c).

Discussion

In lactating female big brown bats, we found that total body water, neutral lipids (body fat), protein, and five macrominerals (calcium, phosphorus, magnesium, sodium, and potassium) account for an average of 96.5% of total body mass. The remaining 3.5% of body mass is likely attributable to polar lipids (i.e. structural lipids), glycogen, minor constituents, and analytical error (Mead et al. 1986).

Despite limitations on the amount of nutrients that small mammals can deposit (Ofstedal 2000), mobilization of maternal fat stores during lactation has been suggested or described for several small mammals, including bats (Fig. 3; Burnett and Kunz 1982; Kiell and Millar 1980; O'Farrell and Studier 1973, 1976; Pistole 1989; Randolph et al. 1995; Reynolds and Kunz 2000; Stack 1985). The maternal fat depots in big brown bats may act as a reserve for periods of low energy intake or relatively high demand. In the present study, we found that females captured during mid-lactation (week 3) had approximately two-thirds less body fat than those collected during other weeks. A drop in body fat, similar to that described here, can be attributable to low ambient temperatures and high rainfall, preventing lactating females from foraging (Hoying and Kunz 1998; Kunz and Hood 2000; Hood et al. 2002) or an increase in the energy demands of the pup or the maintenance requirements of the mother (Dominey 1984; Racey and Speakman 1987, Ofstedal 2000).

There were no apparent changes in extrinsic variables such as ambient temperature or rainfall that would have dramatically altered maternal food intake or metabolism during mid-lactation. High rainfall can restrict bat foraging, but precipitation was low in 1999, with rainfall 42% and 55% below normal for June and July, respectively (<http://www.ncdc.noaa.gov>) and no days with > 10.0 mm of precipitation before week 4 that may have prevented lactating females from foraging. Ambient temperature was also near average (<http://www.ncdc.noaa.gov>), and thus the torpor

Table 1 Mean age, body mass, and macronutrient mass and composition of lactating big brown bats (*Eptesicus fuscus*) relative to other non-reproductive mammals

	N	Age	Body mass (g)	Dry mass (g)	Fat mass (g)	Protein mass (g)	Water (% FFM ^a)	Fat (% DM ^b)	Protein (% FFD ^c)
Lactating big brown bats									
Week 1	4	Adult	16.0 ± 0.7	5.06 ± 0.29	0.863 ± 0.120	3.38 ± 0.19	72.0 ± 2.3	16.9 ± 1.9	80.5 ± 0.7
Week 2	7	Adult	15.8 ± 0.4	5.56 ± 0.18	0.841 ± 0.057	3.63 ± 0.15	68.4 ± 0.5	15.1 ± 0.8	76.8 ± 1.1
Week 3	4	Adult	14.0 ± 0.4	4.52 ± 0.06	0.235 ± 0.027	3.51 ± 0.10	68.9 ± 0.6	5.2 ± 0.5	81.9 ± 2.3
Week 4	5	Adult	16.4 ± 0.8	5.68 ± 0.28	0.899 ± 0.106	3.83 ± 0.13	69.2 ± 0.5	15.7 ± 1.1	80.0 ± 0.3
Week 5	5	Adult	15.5 ± 0.6	5.41 ± 0.19	0.831 ± 0.075	3.56 ± 0.09	68.8 ± 0.5	15.3 ± 1.0	77.8 ± 0.6
Other non-reproductive mammals									
Laboratory mouse ^d	NA	50–60 days	21.4	7.34	2.19	4.09	73.3	30.1	79.8
Laboratory rat ^e	85	70–320 days	269	105	46.0	48.1	73.4	43.7	81.1
Domestic rabbit ^f	5	180–250 days	2,440	809	200	497	72.9	24.7	81.7
Domestic cat ^g	3	Adult	3,590	1,330	435	703	71.1	32.8	78.8
Harp seal ^h	9	5–30 years	129,000	65,800	39,000	23,400	71.7	59.2	87.1
Domestic pig ⁱ	4	170–250 days	97,500	37,000	18,700	13,800	76.5	50.5	75.3
Gray seal ^j	4	Adult	153,000	66,200	32,900	30,000	72.3	49.6	89.9
Domestic cattle ^k	49	2.3–12.5 years	349,000	–	–	–	71.9	41.7	–

Data for big brown bats is given as mean ± standard error. Units for other species were converted from the original published values to match those in this study. Standard errors for these values could not be converted or were not available for many of these studies, thus error is not given. All data were not available for all species. Body composition was determined by chemical analysis of whole body composition minus gut contents (or in post-absorptive individuals) for all studies unless otherwise noted. In this and apparently all other studies (although not clear in all cases), body mass is based on mass prior to dissection

^a FFM expressed as a proportion of fat-free mass

^b DM expressed as a proportion of dry mass

^c FFD expressed as a proportion of fat-free dry mass.

^d *Mus musculus*, Bailey, Kitts and Wood (1960)

^e *Rattus norvegicus*, Spray and Widdowson (1950); unclear if gut contents removed

^f *Oryctolagus cuniculus*, Spray and Widdowson (1950); unclear if gut contents removed

^g *Felis catus*, Spray and Widdowson (1950); unclear if gut contents removed

^h *Phoca groenlandicus*, Gales et al. (1994); body mass given as range age 1–30; we based adult mass on the maximum mass in this range

ⁱ *Sus scrofa*, Spray and Widdowson (1950); unclear if gut contents removed

^j *Halichoerus grypus*, Reilly and Fedak (1990); based on whole body composition including stomach contents; means for adults > 8 years of age

^k *Bos taurus*, Ellenberger et al. (1950)

Table 2 Mean mineral mass and composition of lactating big brown bats (*Eptesicus fuscus*) relative to other non-reproductive mammals

	Total mass of mineral in body (in mg)					Total body concentration (mg/g fat free dry mass)				
	Ca	P	Mg	K	Na	Ca	P	Mg	K	Na
Lactating big brown bats										
Week 1	216 ± 30	169 ± 16	9.03 ± 1.13	39.1 ± 3.8	20.9 ± 0.8	50.5 ± 4.5	40.0 ± 2.5	2.13 ± 0.15	9.3 ± 0.6	4.99 ± 0.07
Week 2	241 ± 12	199 ± 7	9.30 ± 0.30	54.7 ± 2.2	23.0 ± 0.9	51.1 ± 2.0	42.2 ± 1.0	1.98 ± 0.05	11.6 ± 0.3	4.88 ± 0.12
Week 3	181 ± 7	131 ± 5	7.47 ± 0.48	47.9 ± 1.1	19.0 ± 0.5	42.2 ± 1.5	30.6 ± 1.1	1.74 ± 0.10	11.2 ± 0.2	4.44 ± 0.12
Week 4	238 ± 20	184 ± 10	9.33 ± 0.61	56.7 ± 7.6	23.8 ± 1.0	49.4 ± 2.2	38.4 ± 0.8	1.94 ± 0.06	11.8 ± 1.3	4.98 ± 0.09
Week 5	255 ± 25	175 ± 12	9.19 ± 0.26	43.7 ± 2.4	21.4 ± 1.0	55.4 ± 4.3	38.1 ± 1.6	2.01 ± 0.03	9.5 ± 0.3	4.69 ± 0.20
Other non-reproductive mammals										
Laboratory rat ^a	2,720	1,300	61.1	853	396	45.8	21.8	1.03	14.4	6.7
Domestic rabbit ^b	26,300	12,500	852	7,800	3,530	43.1	20.5	1.40	12.8	5.8
Domestic cat ^c	41,300	23,000	1,100	12,400	5,080	46.3	24.7	1.10	13.9	5.7
Domestic pig ^d	914,000	465,000	23,700	270,000	132,000	49.9	25.4	1.29	14.7	7.2
Domestic cattle ^e	—	—	—	—	—	73.8	38.5	—	—	—

Data for big brown bats is given as mean ± standard error. Units for other species were converted from the original published values to match those in this study. Standard errors for these values could not be converted or were not available for many of these studies, thus error is not given. All data were not available for all species. Body composition was determined by chemical analysis of whole body composition minus gut contents (or in post-absorptive individuals) for all studies unless otherwise noted. Note: Sample size, animal mass, dry matter and fat content for all species are presented in Table 1. These values may be used by readers for unit conversion

^a *Rattus norvegicus*, Spray and Widdowson (1950); unclear if gut contents removed

^b *Oryctolagus cuniculus*, Spray and Widdowson (1950); unclear if gut contents removed

^c *Felis catus*, Spray and Widdowson (1950); unclear if gut contents removed

^d *Sus scrofa*, Spray and Widdowson (1950); unclear if gut contents removed

^e *Bos taurus*, Ellenberger et al. (1950)

patterns of lactating females should not have been atypical.

During the third week of lactation, pups first begin taking test flights within the roost while remaining dependent on their mothers for nourishment. Thus, the energy demands of the young may be particularly high at that time (Hood 2001). Lactating females are known to spend more time foraging than pregnant females (Wilkinson and Barclay 1997). Long foraging bouts

coincide with an increase in the cost of milk synthesis and output during mid-lactation, as the fat content of milk increases from early to mid-lactation (Hood 2001). Thus, loss of maternal body fat is likely associated with a reallocation of fat reserves to support milk production and foraging effort.

The apparent rapid recovery from fat depletion during late-lactation is particularly interesting. Although it is possible that low body fat during week 3

Table 3 Mass and mean concentrations of macronutrients and ash in lactating big brown bats (*Eptesicus fuscus*) as presented by Stack (1985)

Week	As presented by Stack (1985)				Estimated from Stack (1985)		
	Body mass (g)	Fat-free dry mass (g)	Fat mass (g)	Ash-free fat-free dry mass (g)	Water (% FFM ^a)	Fat (% DM ^b)	Ash (% FFDM ^c)
1	17.27 ± 0.32	4.30 ± 0.10	1.71 ± 0.12	3.56 ± 0.08	72.4	28.5	17.1
2	16.04 ± 0.24	4.15 ± 0.10	0.98 ± 0.08	3.43 ± 0.08	72.4	19.1	17.4
3	14.74 ± 0.30	3.89 ± 0.12	0.71 ± 0.08	3.24 ± 0.10	72.3	15.4	16.7
4	16.59 ± 0.40	4.03 ± 0.09	1.15 ± 0.07	3.38 ± 0.08	73.9	22.2	16.1
5	17.69 ± 0.47	4.21 ± 0.12	1.62 ± 0.13	3.56 ± 0.10	73.8	27.8	15.4
6	17.15 ± 0.26	4.09 ± 0.11	1.54 ± 0.12	3.45 ± 0.11	73.8	27.4	15.7

Data are expressed by week of lactation. Columns on the left are presented means ± standard errors as reported by Stack (1985). Columns on the right are means estimated from Stack (1985)

^a FFM expressed as a proportion of fat-free mass

^b DM expressed as a proportion of dry mass

^c FFDM expressed as a proportion of fat-free dry mass

Table 4 Mean body mass and macronutrient mass and composition in non-reproductive female bats

	N	Body mass (g)	Dry mass (g)	Fat mass (g)	Fat-free dry mass (g)	Water (% FFM ^a)	Fat (% DM ^b)
Pteropodidae							
<i>Dobsonia praedatrix</i> ^c	2	160	48.6	2.39	46.2	70.7%	4.9%
<i>Macroglossus minimus</i> ^c	5	13.9	4.51	0.50	4.01	70.1%	11.0%
<i>Rousettus amplexicaudatus</i> ^c	20	51.5	16.4	3.09	13.3	72.6%	18.9%
Emballonuridae							
<i>Rhynchonycteris naso</i> ^c	2	4.63	1.64	0.20	1.45	67.4%	11.9%
<i>Saccopterynx bilineata</i> ^c	3	9.21	3.14	0.29	2.85	68.1%	9.3%
<i>Saccopterynx leptura</i> ^c	1	4.42	1.51	0.12	1.40	67.7%	8.1%
Phyllostomidae							
<i>Glossophaga soricina</i> ^c	4	9.32	2.94	0.26	2.68	70.4%	8.9%
<i>Phyllostomus hastatus</i> ^c	4	73.2	24.9	1.91	24.3	67.7%	7.7%
Rhinolophidae							
<i>Aselliscus tricuspis</i> ^c	10	3.70	1.25	0.19	1.06	69.9%	15.1%
<i>Hipposideros diadema</i> ^c	11	36.6	12.4	1.56	10.6	69.1%	12.6%
<i>Hipposideros galeritus</i> ^c	3	7.60	2.47	0.38	2.09	71.1%	15.5%
Vespertilionidae							
<i>Eptesicus fuscus</i> ^d	26	14.3	6.46	—	—	—	17.3%
<i>Myotis lucifugus</i> ^e	13	6.76	2.44	0.29	2.15	66.7%	12.1%
<i>Myotis thysanodes</i> ^e	21	7.57	2.75	0.33	2.43	66.2%	11.8%

Some units were converted from the original published values to match those in this study

Standard errors for these values could not be converted or were not available for many of these studies, thus error is not given. All data were not available for all species. Body composition was determined by chemical analysis of whole body composition minus gut contents (or in post-absorptive individuals) for all studies unless otherwise noted. Although not clear in all cases, body mass is based on mass prior to dissection (i.e. with gut contents if not post absorptive). Note: Although we use common names throughout the rest of the text, we have chosen to use scientific names for bat species here to avoid confusion associated with overlap in common names of bats

^aFFM expressed as a proportion of fat-free mass

^bDM expressed as a proportion of dry mass

^cStudier et al. (1994b); It is unclear if analyses included gut contents

^dPistole (1989); Includes those animals collected early March to early May

^eO'Farrell and Studier (1976); Composition apparently includes gut contents but does not include spleen or adrenal glands

and apparent rapid recovery during week 4 is an artifact of our cross-sectional approach to assigning week of lactation, a drop in body mass from early to mid-lactation and subsequent recovery before weaning was common to lactating big brown bats that were recaptured repeatedly at colonies in Massachusetts and New Hampshire during 1996, 1997, and 1998 (Hood, unpublished data; also see Burnett and Kunz 1982). Many large mammals lose body mass during lactation as body stores partially supplement, or in some cases completely support, the demands of milk production (Bowen et al. 2001; Harlow et al. 2002; Lederman 2004; McNamara and Pettigrew 2002; Oftedal 2000). In smaller species, continuous depletion of body stores may be less common. In many litter-bearing rodents, females gain mass, most likely from body fat, during early lactation and then lose mass in late lactation, presumably supplementing intake when the demands of the litter are highest (Humphries and Boutin 1996;

Kenagy et al. 1989; König et al. 1988; Millar 1978; Sikes 1995).

Fat stores appear to recover in several species of bats following lactation (Fig. 3b), but the onset of recovery can only be evaluated from one other study that examined body composition at multiple points during lactation in bats (Stack 1985). The latter study, which also focused on big brown bats, found a similar pattern of fat loss at mid-lactation with a subsequent recovery (Stack 1985; Table 3). This suggests that fat loss and recovery prior to weaning may be common in this species. The fourth and fifth weeks of lactation are concurrent with the first foraging flights of pups (Hood et al. 2002). Because these pups supplement their milk diet with insects, it is possible the pups' demand for energy from their mothers decreases, allowing mothers to rapidly replenish their fat reserves. Several studies have reported that female big brown bats use torpor while lactating (Audet and

Table 5 Mean mineral mass and composition in non-reproductive female bats

	Total mass of mineral in body (in mg)				Total body concentration (% FFDM ^a)			
	Ca	Mg	Na	K	Ca	Mg	Na	K
Pteropodidae								
<i>Dobsonia praedatrix</i>	1370	63.1	245	517	29.6	1.37	5.31	11.2
<i>Macroglossus minimus</i>	69.7	4.04	19.5	41.2	17.4	1.01	4.86	10.3
<i>Rousettus amplexicaudatus</i>	316	15.4	68.8	147	23.9	1.16	5.19	11.1
Emballonuridae								
<i>Rhynchonycteris naso</i>	17.7	1.29	6.28	12.3	12.2	0.892	4.34	8.50
<i>Saccopterynx bilineata</i>	57.7	3.07	14.0	25.4	20.3	1.08	4.92	8.92
<i>Saccopterynx leptura</i>	22.8	1.20	5.61	12.5	16.2	0.860	4.03	9.00
Phyllostomidae								
<i>Glossophaga soricina</i>	37.2	2.68	14.1	33.0	13.9	1.00	5.25	12.3
<i>Phyllostomus hastatus</i>	454	28.1	100	209	19.7	1.22	4.35	9.07
Rhinolophidae								
<i>Aselliscus tricuspidatus</i>	20.8	1.13	5.66	11.7	19.7	1.07	5.35	11.7
<i>Hipposideros diadema</i>	260	11.6	54.8	109	24.0	1.07	5.07	10.9
<i>Hipposideros galeritus</i>	33.4	2.00	10.6	23.6	16.0	0.960	5.06	11.3

All data are adapted from Studier et al. (1994b)

Units were converted from the original published values to match those in this study. Standard errors for these values could not be converted or were not available for many of these studies, thus error is not given. Body composition was determined by chemical analysis of post-absorptive animals.

Note: Sample size, animal mass, dry matter, fat content, and fat-free dry mass is presented for all species in Table 5. These values may be used by readers for unit conversion.

Note: Although we use common names throughout the rest of the text, we have chosen to use scientific names for bat species here to avoid confusion associated with overlapping in common names of bats

^aFFDM expressed as a proportion of fat-free dry mass

Fenton 1988; Grinevitch et al. 1995; Hamilton and Barclay 1994). It is possible that rate of fat deposition is enhanced by using torpor to lower maintenance requirements. The relationship between the metabolic

demands of milk production and the use of torpor during lactation warrants further investigation. It seems unlikely that the observed loss of body fat during lactation would impair future survival or

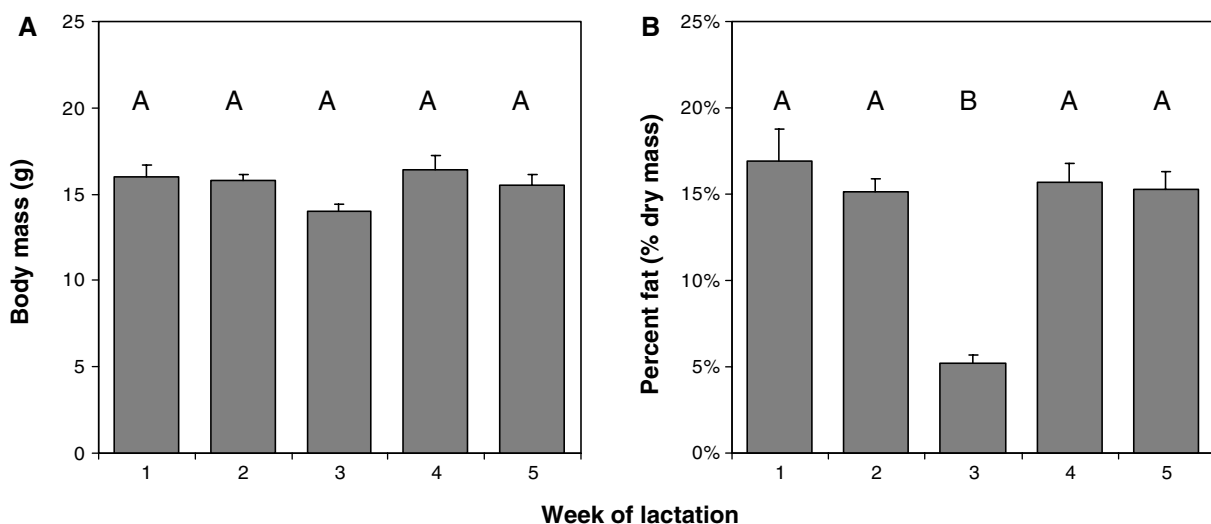


Fig. 1 Variation in maternal body mass (a) and fat (b) concentration during lactation in adult female big brown bats (*Eptesicus fuscus*). Data are expressed as mean \pm standard error.

Letters above bars indicate results of Scheffe's test with significant differences represented by different letters. Sample sizes were 4, 7, 4, 5, and 3 by week, respectively

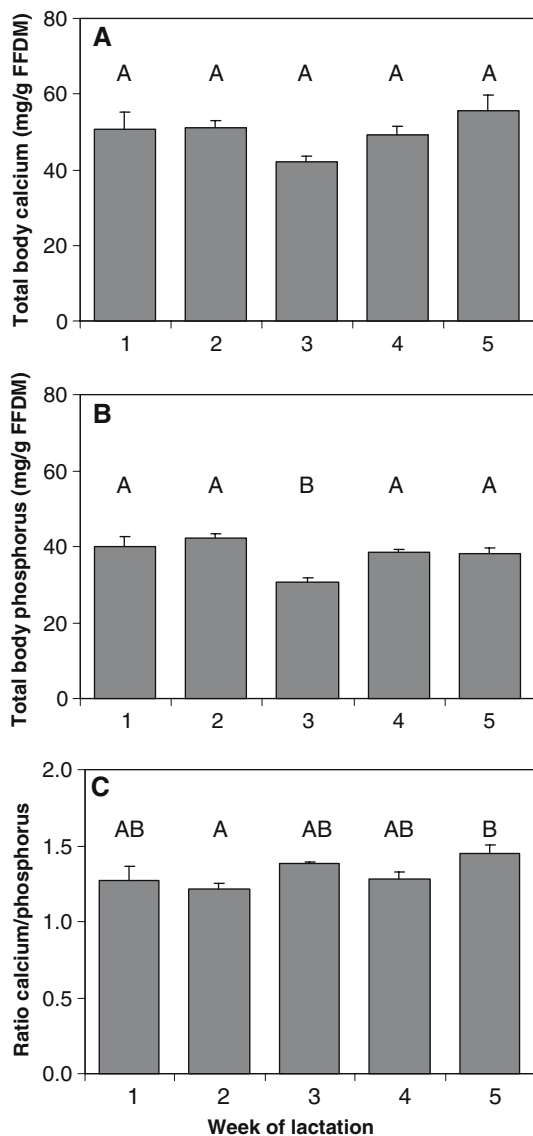


Fig. 2 Variation in maternal calcium (a) phosphorus (b) and ratio of calcium to phosphorus (c) during lactation in adult female big brown bats (*Eptesicus fuscus*). Data are expressed as mean \pm standard error. Phosphorus and calcium are expressed as a function of fat free dry mass (FFDM). Letters above bars indicate results of Scheffe’s test with significant differences represented by different letters. Sample sizes were 4, 7, 4, 5 and 3 by week, respectively

reproductive success. Crude lipid stores had recovered by the end of lactation and there is a several month post-reproductive period of fat deposition that occurs in big brown bats (Pistole 1989) and other temperate hibernating species (Kunz et al. 1998).

Marked demineralization occurs during lactation in the little brown myotis (*Myotis lucifugus*). Kwiecinski et al. (1987) and several other investigators have suggested that this pattern of bone loss indicates that calcium utilization exceeds consumption and may be an important limitation on lactation. Such a calcium

limitation may ultimately constrain litter size in insectivorous bats (Studier et al. 1991, 1994a; Studier and Sevick 1992; Barclay 1994, 1995). In big brown bats, we also observed a significant decrease in phosphorus and an apparent tendency for calcium to decline during week 3 of lactation. Release of phosphorus and calcium from bone is mediated by parathyroid hormone in response to low circulating calcium (Goodman 1994). Although it is possible that loss of phosphorus is associated with loss from other sites of deposition such as soft tissue, we observed no loss of muscle mass, as indicated by the constant crude protein concentration. The calcium to phosphorus ratio was slightly lower during week 2 and elevated during week 5. The small difference in calcium to phosphorus ratio that we observed is not likely to be of biological significance but may reflect relatively small sample size and thus relatively high individual variation in calcium concentration.

In humans, bone demineralization occurs during lactation independent of nutrient intake associated with normal homeorhetic changes that occur during milk production (Prentice 2000). Bone loss is individually variable, but rarely exceeds 10% in mothers that consume the recommended daily intake of calcium and phosphorus (Prentice 2000). Lactation is more intense in small mammals than in humans (Ofstedal 1984) and likewise, the strain on skeletal maintenance is greater. Lactating laboratory rats, suckling an average of seven young, export nearly as much calcium each day as an average woman caring for a single offspring, 0.2 g per day in rats vs. 0.3–0.5 g per day in humans. Despite a four-fold increase in food consumption and a doubling of total calcium absorption, rats lose 5–30% of their calcium stores while lactating (Kovacs 2005). Similarly, laboratory mice, that also bear large litters, lose 20–30% of bone density during lactation (Vanhouten and Wysolmerski 2003). Lactating big brown bats, bearing twins, transfer 0.008 to 0.01 g of calcium to their young each day through milk export (W.R. Hood, unpublished), exceeding the estimated loss of calcium from the skeleton in rats by almost three fold (big brown bat: 0.008 g calcium lost per day/1.28 g skeletal mass = 0.6%, rat: 0.03 g calcium lost per day/12.9 g skeletal mass = 0.2%; Barclay 1994; Kovacs 2005, Prange et al. 1979). Interestingly, the concentrations of both phosphorus and calcium in big brown bats return to early-lactation values by the end of lactation and thus are not likely to affect future reproduction adversely.

We compared the body composition of female big brown bat mothers during lactation with other reproductively active and non-reproductive bats and other mammals (detailed reproductive values were

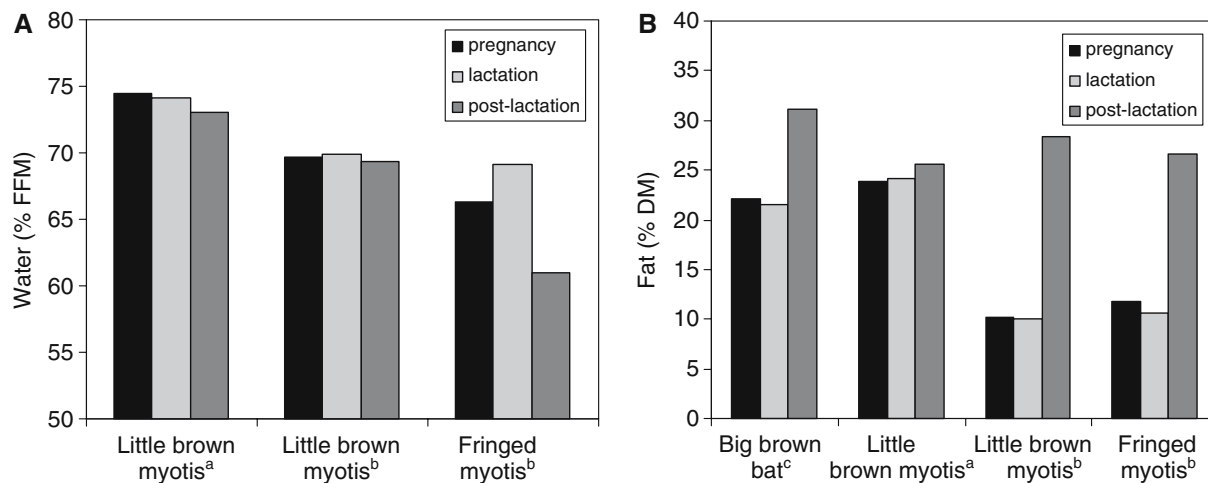


Fig. 3 Variation in total body water (**a**) and total body fat (**b**) in little brown myotis (*Myotis lucifugus*), fringed myotis (*Myotis thysanodes*), and big brown bats (*Eptesicus fuscus*) during pregnancy, lactation, and post-lactation. Data are adapted from previously published work, *a* Reynolds and Kunz 2000 (pregnancy $n = 7$, lactation $n = 12$, post lactation $n = 1$), *b* O'Farrell

and Studier 1976 (Little brown myotis pregnancy $n = 38$, lactation $n = 18$, post lactation $n = 15$; Fringed myotis pregnancy: $n = 30$, lactation $n = 9$, post lactation $n = 51$), and *c* Pistole 1989 (pregnancy $n = 6$, lactation $n = 8$, post lactation $n = 8$)

unavailable for most other species and we did not collect non-reproductive bats), but we did so with caution, as methods of analysis used in the various studies differed substantially. Water is thought to comprise approximately 72% of fat free mass in most mammals, including those presented in Table 1 (Widdowson and Dickerson 1964). Yet, in the present study and in studies of 15 other bat species (Table 4, Fig. 3a), fat-free total body water was slightly below this average. Interestingly, both the two studies on bats that present water contents close to 72% (Stack 1985, Reynolds and Kunz 2000) used a mixture of petroleum ether and ethyl alcohol that would extract neutral and polar lipids, and some glycogen, whereas the present study and all previous studies on bats used petroleum ether that would extract only neutral lipids. These differences in the method of lipid extraction will not only increase the measurements of total body fat but, by reducing fat-free mass (calculated by difference), will cause the ratio of water:fat free mass and protein:fat-free mass to increase.

Differences in hydration status of animals could also influence results among studies. In our study, animals were post-absorptive but were offered water through syringe. Although we cannot preclude that some dehydration could have occurred, concentrations of electrolytes (sodium and potassium) were similar to the range reported for other non-reproductive mammals.

Because skin contains less water relative to other tissues (Widdowson and Dickerson 1964; Stack 1985), it is possible that total body water is generally a lower fraction of fat-free mass in bats than in many other

mammals due to the high skin surface area, associated with large wings. To test this hypothesis, interspecific comparisons are needed using consistent methods of collection, animal hydration, and chemical analyses.

Fat stores in lactating big brown bats were relatively low compared to many non-reproductive mammals (Tables 1, 4). Substantial inter- and intraspecific variation in lipid reserves is not surprising and likely reflects interannual variation in body condition within species and species-specific patterns of lipid storage associated with life history stage. Values of fat composition reported for big brown bats by Stack (1985; Table 3) constituted an approximately 10% greater proportion of dry mass than those observed in the present study. Although differences in methods of extraction may account for some of this variation, the 10% difference observed between our data and Stack's (1985; Mead et al. 1986), suggest that interannual or between colony variation in lipid storage may occur (Hoying and Kunz 1998). This pattern is also indicated by the mean fat content of lactating big brown bats presented by Pistole (1989) who used extraction methods similar to our own study but found an average fat composition that was also greater than 20% of dry mass (Fig. 3b). Interspecific variation in lipid storage between reproductive and non-reproductive animals is likely to reflect seasonal variation in lipid stores as well as different constraints on fat storage associated with mode of locomotion and body size (Oftedal 2000). For example, extensive fat stores are unlikely to hinder locomotion in aquatic species, but may compromise flight ability in volant mammals such as bats (Webb et al. 1992).

Concentrations of calcium in lactating big brown bats are comparable to the non-chiropteran non-reproductive mammals reported in Table 2, except for calcium in dairy cattle. Because the relative mass of the skeleton increases with increasing body mass ($\log \text{ skeletal mass} = 0.061 \times \log \text{ body mass}^{1.09}$; Prange et al. 1979) and calcium is the most abundant element in bone (Spray and Widdowson 1950; Widdowson and Dickerson 1964), it is not surprising that the whole-body calcium concentration in cattle is high. This allometric relationship suggests that smaller species should have lower concentrations of bone minerals than larger species. Calcium and magnesium concentrations in lactating big brown bats are 1.4–4.5 times higher, respectively, than other non-reproductive bats presented by Studier et al. (1994b, phosphorus was not reported, Table 5) but are comparable to non-reproductive terrestrial species that are 17 to 6,000+ times their body mass. Of particular interest are the relatively high concentrations of total body phosphorus and magnesium in lactating bats. In most species, the calcium to phosphorus ratio is approximately 2:1 (Brody 1999), yet the total body ratio of Ca:P in big brown bats was only 1.2:1–1.45:1. It is unclear if the relatively high concentrations of phosphorus are associated with bone (85% of P) or soft tissues (14% of P in most species, Brody 1999). Likewise, it is unclear if relatively high concentrations of magnesium are associated with bone or soft tissues. Because weanling pups have calcium, phosphorus, and magnesium values that approach that of lactating females (Hood 2001), high concentrations of these minerals do not appear to be restricted to lactation. Analysis of the mineral concentrations of individual tissues will be necessary to understand the significance of these values.

High total body concentrations of minerals are associated with an increase in bone density during pregnancy in another small species, the laboratory mouse (Sharpe et al. 2003; Woodrow et al. 2003; Kovacs 2005). Thus, it is possible that some small rodents and bats deposit additional calcium, phosphorus, and magnesium in bone prior to lactation, which could be mobilized during lactation to partially meet the burden of exporting large amounts of calcium relative to their size. Kwiecinski et al. (1987) examined monthly changes in bone density in little brown bats, based on specific gravity and cortical thickness of the humerus. In adult females, the cortex of the humerus was thickest during pregnancy and prior to the onset of hibernation and noticeably thinner following hibernation and again during lactation and post-lactation, with obvious resorption cavities in the alveolar and the cortical bone. The specific gravity of the humerus also

followed these trends, with the annual peak occurring during June pregnancy. Although the specific gravity of the humerus increased prior to hibernation and subsequently declined during hibernation, the pre-hibernation values were not as high as those during pregnancy. Kwiecinski et al. (1987) suggested that the increase in bone density that they observed during pregnancy may be stimulated by increased maternal mass, thus increasing wing loading during pregnancy. Such an increase in bone density would functionally provide lactating females with a reserve of calcium, which may not be necessary to support flight during lactation, when body mass and wing loading is reduced over pregnancy. Low levels of whole-body calcium and phosphorus observed during mid-lactation in big brown bats do not drop below levels typical of other non-reproductive bats (Studier et al. 1994b) and thus concentrations of these minerals may never reach critically low levels.

Patterns of maternal body composition in the big brown bat suggest that lactating females may mobilize some of their own fat stores and bone matrix to support milk production. Lactating females quickly recovered from fat depletion in mid-lactation, which is likely associated with the onset of foraging in young. Whether small litter size in bats has evolved to prevent further depletion of fat and minerals remains unclear. It would be informative to compare nutrient depletion during lactation among females in populations where litter size varies, such as some populations of big brown bats and pallid bats (*Antrozous pallidus*), where some individuals give birth to one young and others produce two. Reduction in phosphorus during mid-lactation in mothers indicates that bone resorption likely occurs. Dependent big brown bat pups from litters of two experienced a simultaneous decline in calcium, phosphorus, and magnesium and subsequent recovery in magnesium (Hood 2001). Although simultaneous reductions in the concentration of minerals in the bone in mothers and pups could be seen as a support for the hypothesis that the uniquely long period of lactation in bats has evolved in concert with low calcium intake (Barclay 1995), high total body calcium and phosphorus suggest that the body may store sufficient calcium and phosphorus to support demands when dietary intake alone is insufficient.

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