Milk composition in a hibernating rodent, the Columbian ground squirrel (*Urocitellus columbianus*)

Author(s): Amy L. Skibiel and Wendy R. Hood
Source: Journal of Mammalogy, 94(1):146-154. 2013.
Published By: American Society of Mammalogists
DOI: http://dx.doi.org/10.1644/1-MAMM-A-078.1
URL: http://www.bioone.org/doi/full/10.1644/1-MAMM-A-078.1

Journal of Mammalogy, 94(1):146–154, 2013

Milk composition in a hibernating rodent, the Columbian ground squirrel (*Urocitellus columbianus*)

Amy L. Skibiel* and Wendy R. Hood

Department of Biological Sciences, Auburn University, AL 36849, USA

* *Correspondent: skibiam@auburn.edu*

Milk is essential to a mammalian mother's reproductive strategy and is necessary for offspring growth and development. In hibernators with a short duration between weaning and winter immergence, milk synthesis is likely constrained by time and trade-offs between maternal and offspring condition, thus influencing milk composition. We characterized the proximate and mineral composition of milk produced by a hibernating rodent, the Columbian ground squirrel (*Urocitellus columbianus*). The concentration of all milk components varied across lactation; the concentration of most constituents peaked between days 14 and 19 postpartum. Columbian ground squirrel milk was relatively low in lipids but high in protein and calcium. At peak lactation, milk was composed of 10.71% ± 0.46% *SE* protein, 9.15% ± 0.47% lipids, 3.39% ± 0.13% sugar, and 0.47% ± 0.02% calcium (wet mass basis). High protein, energy from protein, and calcium in milk corroborate earlier reports of the importance of fast growth rates of juveniles to overwinter survival, whereas the low lipid content of milk may reflect fat conservation for adults. Production of high-calcium milk also may be a preventive mechanism enabling offspring to cope with bone mineral loss during hibernation.

Key words: lactation, mineral composition, proximate composition and temporal variation of milk

DOI: 10.1644/1-MAMM-A-078.1

Lactation is a unique mammalian characteristic that has evolved through numerous physiological and morphological adaptations that support the postpartum production of milk. For example, lactating females display hormonal changes that increase food intake and promote hypertophy of intestinal epithelial cells, which promote greater absorption of ingested nutrients. These adaptations increase the amount of metabolizable nutrients that a lactating mother has available, much of which will be partitioned to milk production (Hammond 1997). Consisting of water, lipids, sugars, and protein (Oftedal 1984), and to a lesser extent, vitamins, minerals, and other biologically active compounds, milk is highly digestible (Robbins et al. 1981) and supports young from parturition to independence (Peaker 1977). Because the nutritional building blocks used to produce milk must be obtained, metabolized, and synthesized by the mother, milk production can come at a high metabolic cost to the female (Gittleman and Thompson 1988). Although costly, milk is imperative for offspring growth and survival; thus, milk components are likely under strong selection pressure (Derrickson et al. 1996) and milk production may be one of the best measures of reproductive effort for mammalian mothers (Festa-Bianchet et al. 1994; Stern et al. 1997).

The nutritional composition of milk represents a balance between providing adequate nutrition for offspring while concomitantly minimizing maternal costs associated with milk synthesis (Milligan et al. 2008). The trade-off between maternal costs of milk production and fitness benefits of producing a nutrient-rich milk may be particularly pronounced when a female's survival is dependent on her body condition at weaning. One clear example of this is in species that enter hibernation shortly after the reproductive period has ended. Unfortunately, knowledge about the composition of milks produced by hibernating species is virtually nonexistent.

Hibernators that do not cache food during winter rely almost entirely on endogenous adipose stores to support their metabolism during winter (Dark 2005). Preparation for this change in physiological state requires a period of hyperphagia associated with increases in food intake rates and deposition of body fat (Dark 2005; Kenagy 1987; Kenagy and Barnes 1988). In many species, hibernation typically begins several months after the young are weaned and thus females and juveniles may have adequate time available for foraging to enhance condition prior to hibernation. For example, female bats in temperate regions typically wean pups by late summer and enter hibernation by late autumn or early winter (Racey and Entwistle 2000). In contrast, Columbian ground squirrels (*Urocitellus columbianus*) begin hibernation just weeks after weaning occurs (Dobson et al. 1992; Young 1990). Thus, an interaction between milk composition and hibernation strategy may be strong in this species.

The purpose of this paper is to characterize milk composition of the Columbian ground squirrel. This species does not cache food in hibernacula and relies on fat catabolism as the primary energy source during hibernation (Dobson et al. 1992; Murie and Boag 1984; Young 1990). Female squirrels nurse pups for approximately 27 days; during this time the only source of nutrition for pups is through the mother's milk (Murie 1992), and pups are weaned just a few weeks before hibernation begins (Dobson et al. 1992; Young 1990). Thus, pups have little time to forage independently and gain fat mass prior to hibernation. Females that wean heavier juveniles with faster growth rates during lactation have a greater number of young survive their 1st hibernation (Skibiel et al. 2009), indicating that a female can alter offspring survival rates via her lactation behavior or the milk she produces. Because the deposition of adipose tissue and fast growth prior to hibernation is essential for juvenile overwinter survival, the production of high-fat milk should be especially important for this species. High protein concentrations in the milk would facilitate faster growth rates. In addition, many small hibernating mammals lose bone mass during hibernation (McGee-Lawrence et al. 2008), and thus we expect milk minerals associated with skeletal growth (particularly calcium) to be high, providing a potential preventative mechanism for bone loss during hibernation.

We also describe changes in milk composition over the course of the lactation period. In most mammals studied to date, concentrations of milk constituents change throughout lactation; however, the direction and pattern of change varies from species to species (Oftedal 1984; Oftedal and Iverson 1995). Typically fat and protein increase while sugar content decreases; however, there are many exceptions to this general trend (Oftedal 1984). For example, in the house mouse (*Mus musculus*), milk fat concentration increases whereas there is no change in protein throughout lactation (Knight et al. 1986). In the yellow-pine chipmunk (*Tamias amoenus*), milk fat and protein content increase whereas sugar content does not vary much from early to late lactation (Veloso et al. 2003). Because fat and protein and minerals associated with skeletal development are likely to be important to Columbian ground squirrel pups, we expect the concentrations of these components to increase throughout lactation.

MATERIALS AND METHODS

Field and laboratory methods.—A population of Columbian ground squirrels was studied on Meadow Dot (50°38′59.6″N, 114°39′40.9″W; elevation 1,565 m) in Sheep River Provincial Park in Alberta, Canada, in 2009. All animals were captured within 2 days following spring emergence from hibernation using Tomahawk live traps (Tomahawk #201 Collapsible Chipmunk Trap, 48 × 15 × 15 cm; Tomahawk Live Trap Co., Tomahawk, Wisconsin) baited with peanut butter. Animals were given uniquely numbered fingerling ear tags and, to facilitate observation from a distance, the dorsal pelage of each individual was painted with a unique dye marking (Lady Clairol Hydrience #51 Black Pearl; Proctor and Gamble, Stamford, Connecticut). Mating dates of females were determined by examining reproductive morphology and by observations of mating behavior.

All females that mated were captured 2–3 days before the estimated parturition date (24-day average gestation—Murie and Harris 1982; Shaw 1925) and transported to the field laboratory to give birth. Following this method, the exact date of parturition was known for all individuals. Females were maintained in individual polycarbonate microvent rat cages ($267 \times 483 \times 20$ mm^3; Allentown Caging Equipment Company, Allentown, New Jersey) filled with pine-chip bedding and strips of newspaper for nesting material and covered in vented black plastic bags. Squirrels were fed a diet of show-horse feed (oats, barley, wheat, and compressed vegetable material in a molasses mix; EQuisine; Unifeed, Okotoks, Alberta, Canada) given ad libitum and lettuce and apple twice daily. Cages were monitored for pups 3 or 4 times per day between 0700 and 2200 h. Mother and pups were released back on the field 1–2 days following parturition by opening the cage and allowing the mother to retrieve her pups and take them to her nest burrow.

In our population, juveniles emerged from the nest burrows on average 27 days ± 0.31 *SE* postpartum. From birth to litter emergence the only source of nutrition for pups is through milk consumption (Murie 1992) and emergence from nest burrows approximates the time of weaning (Andersen et al. 1976; Murie and Dobson 1987). Milk was collected from reproductive females ($n = 36$) on day 4, 9, 14, 19, and 25 postpartum (day of birth = day 0). Eight of these females lost their litter during the lactation period, leaving 28 females for which we have milk samples from all 5 collection days. Females were captured in the field and held in traps for 3 h before being milked to allow for milk accumulation within the mammary glands. Animals were anesthetized by placing them in a 4-liter plastic container with a screw-top lid containing a cotton ball presoaked in isoflurane. Oxytocin (concentration = 20 USP/ml, dose = 0.5 μl/g; Osborn; Bimeda Inc., Oakbrook Terrace, Illinois) was administered intramuscularly to stimulate milk letdown. Hair surrounding the nipples was trimmed and the nipples and surrounding area were cleaned with isopropyl alcohol prior to milk expression. Milk was collected from 1–3 glands and each gland was evacuated completely. Milk was expressed by light manual palpation of the mammary gland directly into a glass capillary tube, immediately transferred to screw-top storage vials, and stored on ice until transported to the field station. Samples were then stored at –20°C until they were shipped on dry ice to Auburn University and subsequently stored at –80°C until assaying. All methods used in this study follow guidelines of the American Society of Mammalogists (Sikes et al. 2011) and were approved by the Auburn University Institutional Animal Care and Use Committee (protocol 2009-1151) and the University of Calgary Life and Environmental Sciences Animal Care Committee (protocol BIO 9R-42).

Milk analysis.—Proximate and mineral composition of milk followed methods for small quantities of milk (Hood et al. 2009). Samples were assayed in duplicate or triplicate as allowed by sample volume and concentrations of milk constituents were averaged across replicates. Dry matter content of milk was determined based on change in mass following drying at 100°C for 3 h in a forced convection oven (Binder Drying Oven FED 115-UL; Binder Inc., Great River, New York). The crude protein content of milk was determined based on carbon, hydrogen, and nitrogen (CHN) elemental analysis (Perkin Elmer PE2400 Series II CHNS/O Elemental Analyzer; Perkin Elmer, Shelton, Connecticut). Crude protein was estimated by multiplying total nitrogen in the sample by the standard conversion factor, 6.38 (Jones 1931). Total lipid content was estimated by the Roese–Gottlieb ether extraction method modified for microvolumes of milk (AOAC 1990) and sugar content was measured through the phenol–sulfuric acid method (Dubois et al. 1956; Marier and Boulet 1959). The resulting sugar values were multiplied by 0.95 to correct for hydration of the lactose monohydrate standard used in the assay.

For determination of mineral composition, wet samples were 1st digested in trace-metal–grade nitric acid in a Speedwave MWS-2 Microwave Digester (Berghof Products + Instruments GmbH, Eningen, Germany) with the microwave ramped to 200°C over 15 min, held at 200°C for 15 min, and then ramped back down to room temperature over the final 15 min. Samples were diluted with plasma-grade water (Fisher Chemical, Fair Lawn, New Jersey) and the mineral content (calcium, sodium, potassium, and magnesium) was then determined by inductively coupled plasma–optical emission spectrometry (Perkin Elmer Optima 7300DV; Perkin Elmer, Waltham, Massachusetts). An internal standard (Ag) was added to all samples prior to inductively coupled plasma–optical emission spectrometry analysis to determine recovery of minerals in the samples. Mineral replicates with internal standard recoveries less than 90% were excluded from averages for that sample. All assays were validated using either whole cow's milk (sugar and lipid assays) or powdered milk from the National Institute of Standards and Technology (dry matter, protein, and mineral assays). Recoveries for cow's milk were: sugars = 98% ($n = 40$) and lipids = 92% ($n = 42$). Recoveries for National Institute of Standards and Technology, Gaithersburg, Maryland, milk powder are as follows ($n = 9$ for all assays): dry matter = 98%, protein = 95%, calcium = 111%, potassium = 81%, magnesium = 102%, and sodium = 103%. Thus, estimates of lipid and K concentrations in squirrel milk may be lower than the actual concentration and estimates of Ca concentrations may be slightly higher than the actual concentration. Gross energy (GE) content of milk was estimated from energy (E) equivalents of protein, sugars, and fat by the equation $E = 9.11F + 5.86P + 3.95L$, where the units for fat (F), protein (P), and sugars (L) are grams per gram of whole milk as in Derrickson et al. (1996).

Statistics.—All statistical analyses were conducted in SAS (SAS Institute Inc. 2002). Normality of data was visualized graphically and determined statistically through Shapiro–Wilks tests. All data were normally distributed with the exception of milk calcium and potassium concentrations with calcium having a slight left skew and potassium having a slight right skew. Transformations did not improve normality so analyses were performed with untransformed data. Separate mixed models (PROC MIXED) were used to test effects of lactation stage on each milk constituent. Female identification was included as a random effect. Day of sample collection was included as a predictor variable as well as day squared to account for potential curvilinear relationships. These predictor variables are within-subject effects and thus our model does not include any between-subject effects. Because milk composition may vary with litter size (Fiorotto et al. 1991), we included litter size at birth in the models. Litter sizes did not have a significant effect on any milk component ($P > 0.1$ for all models) and thus, litter size was excluded from all models examining changes in milk composition over time. A Bonferroni correction was applied to adjust the significance level of each model for multiple comparisons. We ran 12 analyses on different milk constituents therefore the α-level was adjusted to 0.004, which was calculated by dividing the α-level of 0.05 by the number of comparisons. Data are presented as means ± *SE*.

RESULTS

At peak lactation (day 19 postpartum), the dry matter content of Columbian ground squirrel milk was 29.9% ± 0.97% of wet mass. Protein and lipids were the largest proximate constituents in the milk at 10.7% ± 0.46% and 9.15% ± 0.47%, respectively. Milk consisted of 3.39% ± 0.13% sugars. Of the mineral components, calcium occurred in higher concentrations than potassium, sodium, and magnesium (calcium: 0.47% ± 0.02%, potassium: 0.14% ± 0.006%, sodium: 0.09% ± 0.003%, magnesium: 0.02% ± 0.001%). Milk energy at peak lactation was 1.60 ± 0.05 kcal/g. Percent of milk energy derived from protein, lipids, and sugars was 39.63% ± 1.53%, 51.61% ± 1.51%, and 8.76% ± 0.54%, respectively.

The concentration of most milk components varied over the course of the lactation period (Table 1) and many constituents showed a distinct peak between 14 and 19 days postpartum (Figs. 1 and 2). Of the components assayed, only concentrations of lipids, sugar, and sodium, and the proportion of energy from lipids were constant over time (Table 1). Dry matter, protein, and energy exhibited similar patterns of change throughout lactation. Dry matter increased from 24% of wet mass to approximately 30% at 19 days postpartum followed by a slight decrease to 27% in late lactation (Fig. 1) and protein rose from 7% wet mass to 11% and then decreased to 10% by

TABLE 1.—Results of mixed models for characterizing changes in the proximate and macromineral concentration and energy content of Columbian ground squirrel milk from parturition to weaning.

	Proximate			
	Predictor[a]	*d.f.*	*F*-value	*P*-value
Milk constituent				
Dry matter	Day	1, 30	29.71	<0.0001*
	Day^2	1, 28	23.48	<0.0001*
Lipids	Day	1, 30	4.31	0.04
	Day^2	1, 28	6.12	0.02
Protein	Day	1, 30	57.25	<0.0001*
	Day^2	1, 28	38.64	<0.0001*
Sugar	Day	1, 30	0.01	0.93
	Day^2	1, 28	4.49	0.04
Minerals				
Calcium	Day	1, 30	69.83	<0.0001*
	Day^2	1, 28	38.15	<0.0001*
Magnesium	Day	1, 30	33.87	<0.0001*
	Day^2	1, 28	26.44	<0.0001*
Potassium	Day	1, 30	16.96	0.0003*
	Day^2	1, 28	18.74	<0.0002*
Sodium	Day	1, 30	6.20	0.02
	Day^2	1, 28	0.39	0.54
Energy				
Gross energy (GE[b])	Day	1, 30	27.13	<0.0001*
	Day^2	1, 28	20.75	<0.0001*
GE protein	Day	1, 30	15.98	0.0004*
	Day^2	1, 28	7.76	0.01
GE lipids	Day	1, 30	8.23	0.008
	Day^2	1, 28	1.45	0.24
GE sugars	Day	1, 30	8.39	0.007
	Day^2	1, 28	16.52	0.0004*

[a] Day refers to day of sample collection and day squared also is included in statistical analyses to account for potential curvilinear relationships.

[b] The proportions of gross energy (GE) from proximate components are given as GE protein, lipids, and sugars.

* Significance at an α-level of 0.004 (see "Materials and Methods").

25 days postpartum (Fig. 1). Gross energy of milk increased from 1.3 kcal/g to 1.6 kcal/g at 19 days postpartum and then decreased to 1.5 kcal/g at late lactation (Fig. 1). Composition of milk calcium changed most dramatically, with calcium increasing by 134% from early to peak lactation and then decreasing by 8% by 25 days postpartum (Fig. 2). The proportion of milk energy from sugars was fairly level until peak lactation and then rose to late lactation, whereas the proportion of energy from protein showed the opposite pattern of change (Table 1; Fig. 3).

DISCUSSION

Reproduction is constrained by time and energy requirements in hibernating mammals (Heaney 1984) and thus is likely to dictate maternal investment in reproduction, particularly for species in which females and juveniles have a short amount of time postweaning to accumulate fat stores prior to hibernating (Phillips 1984). In Columbian ground squirrels, females and juveniles only have a few weeks postweaning to increase condition (Dobson et al. 1992; Young 1990) and juveniles that are weaned at a heavier mass and grow faster during the lactation period are more likely to survive their 1st hibernation (Skibiel et al. 2009). For this reason we expected females to produce milk high in fat, protein, and energy, which would result in heavier and larger young at weaning.

Although there are insufficient data to apply statistical tests for comparison of milk produced by our study species to other rodents and hibernating mammals, qualitative comparisons suggest that dry matter, sugar concentration, and energy content of Columbian ground squirrel milk at peak lactation (i.e., midlactation, 19 days postpartum) is comparable to reports for other rodents and hibernating mammals (Table 2). Although lipids and protein comprised the greatest proportion of wet mass at approximately 9% each, lipid concentration in Columbian ground squirrel milk at midlactation is relatively low compared to that of other rodents. For example, house mice and deer mice (*Peromyscus maniculatus*) have 27% and 21% lipids, respectively, at midlactation (Derrickson et al. 1996; Oftedal and Iverson 1995; Table 2). Lipid concentration in Columbian ground squirrel milk is up to 20% lower than in hibernating vespertilionid bats (Kunz et al. 1995) and is approximately 13% lower in lipids than the yellow-pine chipmunk, another hibernating rodent (Veloso et al. 2003; Table 2). In addition, the proportion of energy from lipids was relatively low. This is surprising considering that Columbian ground squirrels do not feed during the hibernation period and thus rely solely on endogenous fat reserves to meet energy needs (Dobson et al. 1992; Murie and Boag 1984; Young 1990). Although yellow-pine chipmunks increase fat mass prior to hibernation (Geiser and Kenagy 1987), they also store food for use during winter (Kenagy and Barnes 1988; Schulte-Hostedde et al. 2001) and thus may not face the same demands for mass gain as Columbian ground squirrels.

The relatively low lipid concentration found in Columbian ground squirrel milk relative to other hibernators may be attributed to several factors. First, it may be indicative of the importance of fat conservation for adult females in this species. Second, it may reflect the ability of juveniles to compensate for low-fat milk through food consumption postweaning. Juvenile Columbian ground squirrels typically enter hibernation by the beginning of September, whereas adult females enter their 1st torpor in mid-August (Young 1990). This 2-week difference in torpor entry by juveniles may afford them a sufficient amount of time to gain fat reserves prior to hibernating. Third, fatty acid composition of milk lipids may be more important for juvenile hibernators than total amount of fat consumed. Unsaturated fatty acids, particularly the essential unsaturated fatty acids such as linolenic and linoleic acids, appear to be especially important for hibernators because sciurids fed diets high in these acids had longer torpor bouts and lower body temperatures during hibernation, resulting in substantial energy savings (Florant et al. 1993; Frank 1992). Fourth, the low lipid concentration of Columbian ground squirrel milk may be related to body size. Small mammals, such as shrews (mass range from 3 to 18 g—Nowak 1999), are limited in their ability to store fat to support lactation because of constraints on body size (Oftedal 2000). Likewise, body size is inversely correlated

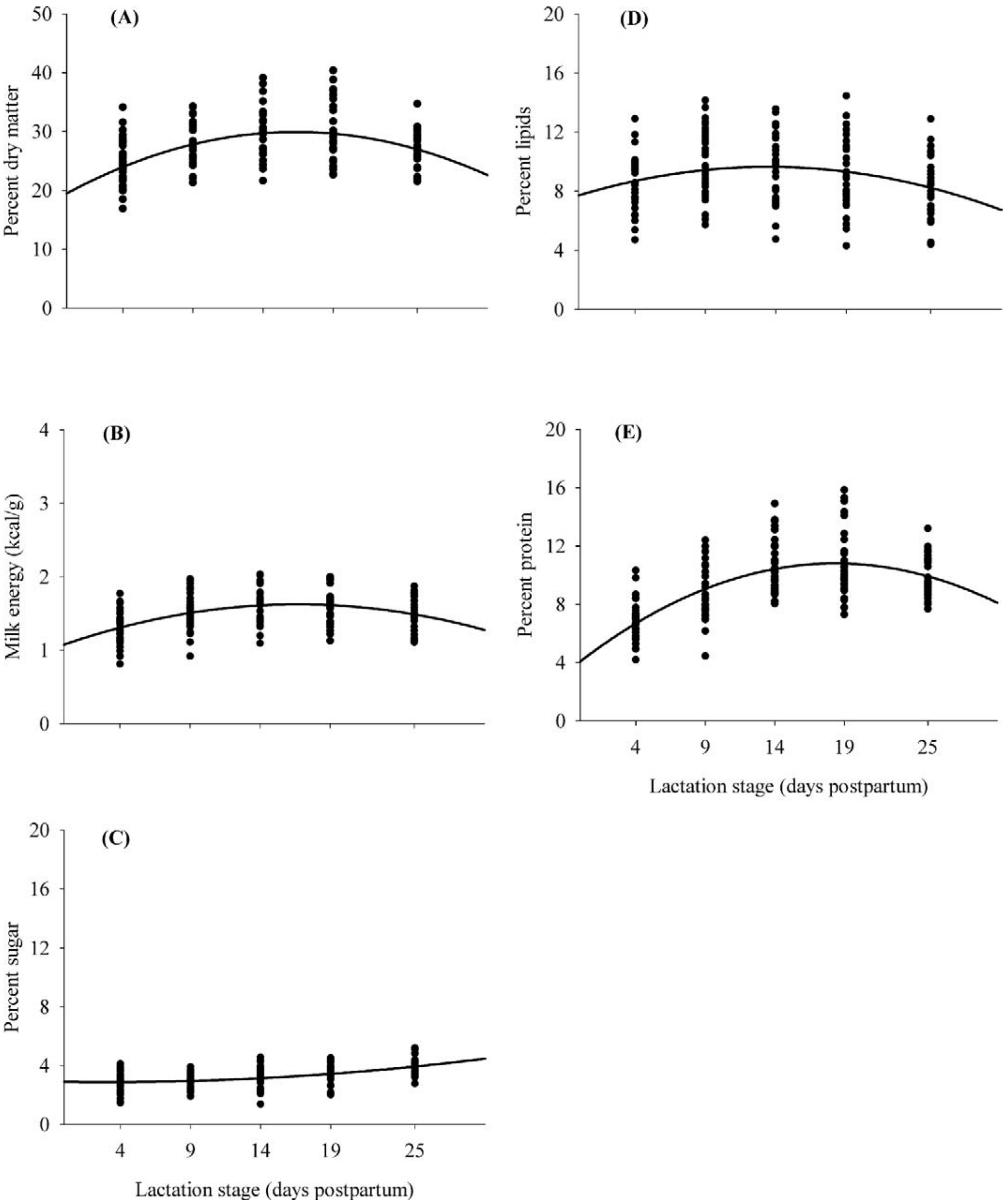

Fig. 1.—Temporal changes in the concentration of proximate milk components, dry matter, and gross energy: A) change in dry matter composition, B) change in gross energy content, C) change in sugar concentration, D) change in lipid concentration, and E) change in protein concentration.

with the concentration of milk dry matter and fat (Derrickson et al. 1996), suggesting that there also are constraints on the volume of milk that small mammals can carry. This likely occurs because larger volumes of milk increase body mass resulting in lower locomotor agility. In bats, more concentrated milk may have evolved to reduce maternal mammary mass and wing-loading, allowing for flight (Kunz et al. 1995). Similarly, for small, nonvolant mammals, highly concentrated milk reduces milk volume, which allows them to maintain agility, whereas larger species, such as Columbian ground squirrels (average 500 g at peak lactation), should not be under the same constraints to reduce volume of milk by producing highly concentrated and high-fat milk.

Protein concentrations in Columbian ground squirrel milk at midlactation were similar to those of other hibernating species and to other rodents, but the proportion of milk energy derived from protein was much higher in Columbian ground squirrels (Table 2). Hibernating vespertilionid bats have between 8.5% and 10.5% protein (Kunz et al. 1995; Table 2) and Columbian ground squirrel milk consists of approximately 11% protein at midlactation. Rodents such as the Norway rat (*Rattus norvegicus*), house mouse, deer mouse, and yellow-pine chipmunk have protein concentrations at midlactation between 8% and 12.5% (Derrickson et al. 1996; Oftedal and Iverson 1995; Veloso et al. 2003; Table 2). Small rodents, such as these, produce young with high growth rates (Case 1978; Reiss

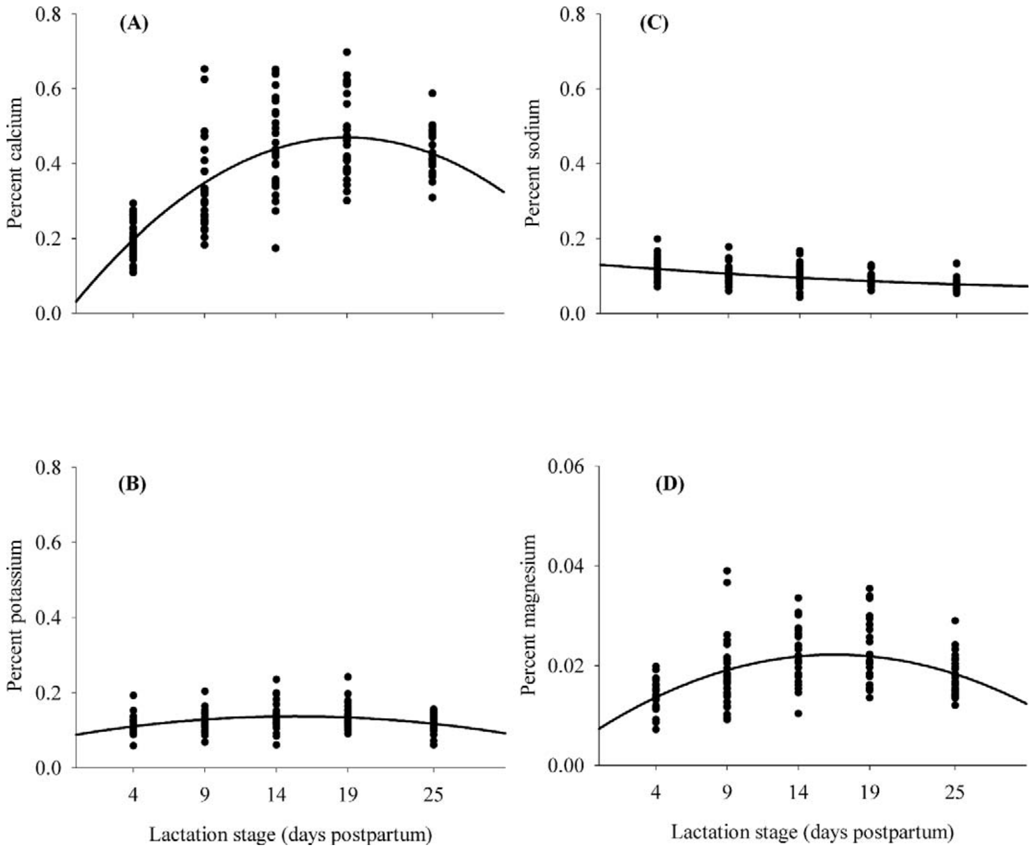

Fig. 2.—Temporal changes in milk mineral composition: A) change in calcium concentration, B) change in potassium concentration, C) change in sodium concentration, and D) change in magnesium concentration.

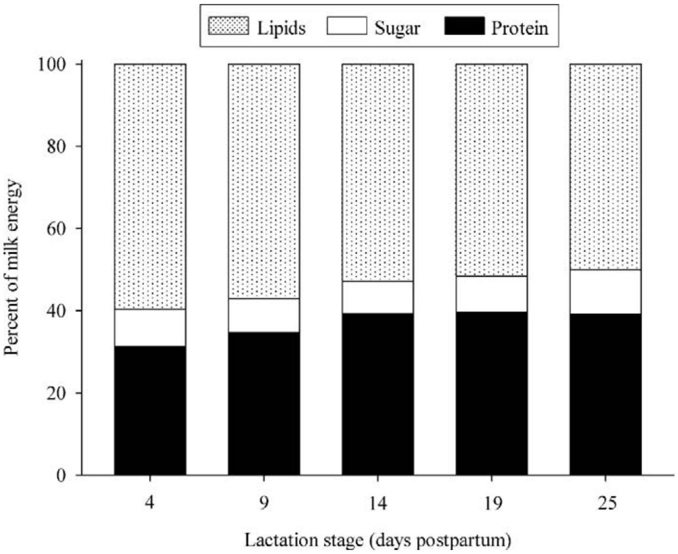

Fig. 3.—Contribution of lipids, protein, and carbohydrates to gross energy content of milk throughout lactation. Gross energy (E) was calculated by the equation $E = 9.11F + 5.86P + 3.95L$, where the units for fat (F), protein (P), and sugars (L) are grams per gram of whole milk (Derrickson et al. 1996).

1989) and thus are expected to produce milk higher in protein concentrations than species such as primates that have slow growth rates (primates range from 1.1% to 5.2% protein at midlactation [Oftedal and Iverson 1995]). In addition, a higher contribution of protein to gross energy is expected in species with faster growth rates. The proportion of milk gross energy from protein in slow-growing primates ranges from 6% to 28% (Hinde et al. 2009; Power et al. 2002), whereas in rodents, it ranges from ~18% to 40% (Table 2). It is interesting that percent gross energy from protein is much higher in Columbian ground squirrel milk than in other species with fast growth rates, suggesting that in this species fast growth is especially important. This corroborates the previous finding of Skibiel et al. (2009) that the number of juveniles surviving hibernation is dependent on growth rate during the lactation period.

Minerals are essential for many physiological and developmental processes. Minerals such as sodium and potassium are involved in osmotic and acid–base balance and calcium, phosphorus, and magnesium are necessary for bone ossification and growth (Barboza et al. 2009; Brody 1999). Calcium concentrations were slightly higher in Columbian ground squirrel milk than in other mammals in general (Studier and Kunz 1995) and other rodents in particular (Table 3). Some empirical evidence suggests that hibernating species, such as ground squirrels, bats, and hamsters, lose bone during

Table 2.—Comparison of the proximate composition of milk (%) and percent energy from proximate constituents (in parentheses) at midlactation among hibernating and nonhibernating rodents and 2 species of bats.

Family	Species	Hibernates?	Dry matter	Sugar	Lipids	Protein	Energy (kJ/g)	Energy[a] (kcal/g)	Reference[b]
Sciuridae	*Urocitellus columbianus*	Yes	29.9	3.4 (8.6)	9.2 (53.4)	10.7 (40.2)	6.5	1.6	1
	Tamias amoenus	Yes	36.7	4.8 (6.7)	23.4 (75.5)	8.5 (17.6)	11.8	2.8	2
Cricetidae	*Peromyscus maniculatus*	No	32.0	2.4 (3.7)	21.0 (74.1)	9.9 (22.5)	10.8	2.6	3
	Microtus montebelli	No	23.3	1.6 (3.7)	11.8 (62.5)	9.9 (33.7)	7.2	1.7	4
Chinchillidae	*Chinchilla lanigera*	No	20.2	1.7 (4.4)	11.2 (67.2)	7.3 (28.2)	6.4	1.5	5
Caviidae	*Kerodon rupestris*	No	19.2	5.3 (16.6)	6.8 (49.6)	6.6 (30.9)	5.2	1.3	3
	Cavia porcellus	No	17.5	4.8 (17.6)	5.7 (48.1)	6.3 (34.2)	4.5	1.1	6, 7
Muridae	*Acomys cahirinus*	No	42.0	2.3 (3.2)	23.0 (75.7)	12.3 (26.0)	11.6	2.8	3
	Mus musculus	No	40.8	2.6 (3.1)	27.0 (74.6)	12.5 (22.2)	13.8	3.3	5
	Rattus norvegicus	No	22.1	3.8 (10.5)	8.8 (56.1)	8.1 (33.2)	6.0	1.4	5
Vespertilionidae (Chiroptera)	*Myotis lucifugus*	Yes	27.1	4.0 (7.6)	15.8 (70.3)	8.5 (24.4)	8.6	2.0	8
	Myotis velifer	Yes	32.4	4.4 (6.8)	19.9 (71.4)	10.7 (24.6)	10.6	2.5	8

[a] Energy content of milk (E; kcal/g) was estimated from energy equivalents of protein, sugars, and fat by the equation $E = 9.11F + 5.86P + 3.95L$, where the units for fat (F), protein (P), and sugars (L) are grams per gram of whole milk as in Derrickson et al. (1996).

[b] 1, current study; 2, Veloso et al. 2003; 3, Derrickson et al. 1996; 4, Sugawara et al. 1990; 5, Oftedal and Iverson 1995; 6, Oftedal 1981; 7, Nelson et al. 1951; 8, Kunz et al. 1995.

hibernation due to an increase in bone resorption and a decrease in bone formation (Haller and Zimny 1977; Kwiecinski et al. 1987; Steinberg et al. 1981). However, more recent research on golden-mantled ground squirrels (*Callospermophilus lateralis*) and thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) found no effect of hibernation on geometrical or mechanical properties of the tibia and femur (McGee-Lawrence et al. 2011; Utz et al. 2009). However, there were microstructural differences between hibernating and active thirteen-lined ground squirrels in both cortical and trabecular bone, which indicate that this species, and potentially other small hibernating mammals, cannot prevent bone loss from disuse entirely during hibernation (McGee-Lawrence et al. 2011). It is possible that elevated calcium concentrations in milk may serve to bolster juveniles against subsequent changes in calcium homeostasis during hibernation. This may be particularly important for Columbian ground squirrels because they do not attain adult structural size until approximately 2 years of age. In addition, because calcium is imperative for bone growth (Barboza et al. 2009; Brody 1999), high amounts of calcium transferred in milk likely also support rapid structural growth of neonates.

For all mammals whose milk composition has been analyzed, proximate or mineral concentration, or both, of milk changes as lactation progresses, but the components that change and the pattern of change vary among species (Oftedal 1984; Oftedal and Iverson 1995). Like other rodents, most proximate and mineral components of Columbian ground squirrel milk changed throughout lactation; however, the pattern of change differed from other species. In Columbian ground squirrel milk, protein, dry matter, calcium, potassium, and magnesium concentrations increased toward peak lactation and then decreased from peak to late lactation rather than increasing linearly from early to late lactation (Figs. 1 and 2). Lipids varied little throughout lactation, which could reflect the importance of lipid acquisition in this hibernating species. As for energy, in many chiropterans and the yellow-pine chipmunk, energy increased from early to late lactation primarily due to an increase in fat (Kunz et al. 1995; Stern et al. 1997; Veloso et al. 2003). Gross energy content of Columbian ground squirrel milk increased to midlactation and then decreased toward late lactation, reflecting the changes in the proportions of milk energy from protein and sugars (Figs. 1 and 3).

In conclusion, dry matter and lactose concentration in Columbian ground squirrel milk was similar to that of bats and rodents, whereas lipid and energy content was lower than expected based on the hibernation strategy of this species. It is possible that mothers prioritize the use of fatty acids for maintaining their own adipose stores over using fatty acids and the building blocks of triacylglycerols for milk synthesis. This could be particularly important given that mothers have

Table 3.—Comparison of milk mineral composition at midlactation for several rodents. Concentrations are given as mg/ml unless otherwise specified.

Family	Species	Ca	K	Mg	Na	P	Reference[a]
Sciuridae	*Urocitellus columbianus*	4.7[b]	1.4[b]	0.23[b]	0.86[b]	3.9[b]	1
Muridae	*Mus musculus*	3.6	1.1	0.24	0.84	2.2	2
	Rattus norvegicus	1.0	—	0.16	—	—	3
Cricetidae	*Microtus montebelli*	2.9	—	—	—	—	4
Caviidae	*Cavia porcellus*	2.3	0.89	0.27	0.47	1.2	5

[a] References: 1, current study; 2, Yajima et al. 2006; 3, Keen et al. 1981; 4, Sugawara et al. 1990; 5, Anderson and Sheffield 1988.

[b] Data are in mg/g (approximately equivalent to mg/ml).

relatively less time between weaning and hibernation than do their young. Alternatively, the low lipid concentration of Columbian ground squirrel milk may be attributed to their size; rapid fat deposition in the days prior to entering hibernation may make it unnecessary for females or their young to have substantial adipose stores at weaning. We also found that protein and the proportion of milk energy from protein were higher in this species than in other rodents and bats, indicating the importance of fast growth rates in this species. Finally, calcium concentration of Columbian ground squirrel milk was relatively high, potentially contributing to rapid neonatal growth or bolstering juveniles against subsequent bone loss during hibernation, or both.

ACKNOWLEDGMENTS

We thank our field and laboratory assistants for assisting with data collection and laboratory assays. M. Power and C. Power conducted the carbon, hydrogen, and nitrogen analyses at the Nutrition Laboratory of the Smithsonian Institution National Zoological Park. Members of the Hill laboratory provided valuable comments on the manuscript. In the field, researchers were housed at the University of Calgary Biogeoscience (BGS) Institute's R. B. Miller Field Station. We recognize E. Johnson, K. Ruckstuhl, and J. Buchanan-Mappin. Funding was provided by the Auburn University College of Science and Mathematics (WRH), Sigma Xi Grants-in-Aid of Research (G2009151040 and G200810150150) awarded to ALS, and a National Science Foundation research grant (DEB-0089473) to F. S. Dobson.

LITERATURE CITED

ANDERSEN, D. C., K. B. ARMITAGE, AND R. S. HOFFMANN. 1976. Socioecology of marmots: female reproductive strategies. Ecology 57:552–560.

ANDERSON, R. R., AND G. G. SHEFFIELD. 1988. Macrominerals in guinea pig milk during 21 days of lactation. Journal of Dairy Science 71:337–345.

AOAC. 1990. Official methods of analyses of the Association of Official Analytical Chemists, 15th ed. Association of Official Analytical Chemists, Inc., Arlington, Virginia.

BARBOZA, P. S., K. L. PARKER, AND I. D. HUME. 2009. Integrative wildlife nutrition. Springer-Verlag, Heidelberg, Germany.

BRODY, T. 1999. Nutritional biochemistry. Academic Press, San Diego, California.

CASE,T. J. 1978. On the evolution and adaptive significance of postnatal growth rates in the terrestrial vertebrates. Quarterly Review of Biology 53:243–282.

DARK, J. 2005. Annual lipid cycles in hibernators: integration of physiology and behavior. Annual Review of Nutrition 25:469–497.

DERRICKSON, E. M., N. JERRARD, AND O. T. OFTEDAL. 1996. Milk composition of two precocial, arid-dwelling rodents, *Kerodon rupestris* and *Acomys cahirinus*. Physiological Zoology 69:1402–1418.

DOBSON, F. S., M. J. BADRY, AND C. GEDDES. 1992. Seasonal activity and body mass of Columbian ground squirrels. Canadian Journal of Zoology 70:1364–1368.

DUBOIS, M., K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH. 1956. Calorimetric method for determination of sugars and related substances. Analytical Chemistry 28:350–356.

FESTA-BIANCHET, M., J. T. JORGENSON, AND W. D. WISHART. 1994. Early weaning in bighorn sheep, *Ovis canadensis*, affects growth of males but not of females. Behavioral Ecology 5:21–27.

FIOROTTO, M. L., D. G. BURRIN, M. PEREZ, AND P. J. REEDS. 1991. Intake and use of milk nutrients by rat pups suckled in small, medium, or large litters. American Journal of Physiology 260:R1104–R1113.

FLORANT, G. L., L. HESTER, S. AMEENUDDIN, AND D. A. RINTOUL. 1993. The effect of a low essential fatty acid diet on hibernation in marmots. American Journal of Physiology 264:R414–R419.

FRANK, C. L. 1992. The influence of dietary fatty acids on hibernation by golden-mantled ground squirrels (*S. lateralis*). Physiological and Biochemical Zoology 65:906–920.

GEISER, F., AND G. J. KENAGY. 1987. Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. American Journal of Physiology 252:R897–R901.

GITTLEMAN, J. L., AND S. D. THOMPSON. 1988. Energy allocation in mammalian reproduction. American Zoologist 28:863–875.

HALLER, A. C., AND M. L. ZIMNY. 1977. Effects of hibernation on interradicular alveolar bone. Journal of Dental Research 56:1552–1557.

HAMMOND, K. A. 1997. Adaptation of the maternal intestine during lactation. Journal of Mammary Gland Biology and Neoplasia 2:243–252.

HEANEY, L. R. 1984. Climatic influences on life history tactics and behavior of North American tree squirrels. Pp. 43–78 in The biology of ground-dwelling squirrels: annual cycles, behavioral ecology, and sociality (J. O. Murie and G. R. Michener, eds.). University of Nebraska Press, Lincoln.

HINDE, K., M. L. POWER, AND O. T. OFTEDAL. 2009. *Rhesus macaque* milk: magnitude, sources, and consequences of individual variation over lactation. American Journal of Physical Anthropology 138:148–157.

HOOD, W. R., M. B. VOLTURA, AND O. T. OFTEDAL. 2009. Methods of measuring milk composition and yield in small mammals. Pp. 529–553 in Ecological and behavioral methods for the study of bats (T. H. Kunz and S. Parsons, eds.). Johns Hopkins University Press, Baltimore, Maryland.

JONES, D. B. 1931. Factors for converting percentages of nitrogen in food and feed into percentages of proteins. United States Department of Agriculture Circular 183. United States Government Printing Office, Washington, D.C.

KEEN, C. L., B. LÖNNERDAL, M. CLEGG, AND L. S. HURLEY. 1981. Developmental changes in composition of rat milk: trace elements, minerals, protein, carbohydrate, fat. Journal of Nutrition 111:226–230.

KENAGY, G. J. 1987. Energy allocation for reproduction in the golden-mantled ground squirrel. Symposia of the Zoological Society of London 57:259–273.

KENAGY, G. J., AND B. M. BARNES. 1988. Seasonal reproductive patterns in four coexisting rodent species from the Cascade Mountains, Washington. Journal of Mammalogy 69:274–292.

KNIGHT, C. H., E. MALTZ, AND A. H. DOCHERTY. 1986. Milk yield and composition in mice: effects of litter size and lactation number. Comparative Biochemistry and Physiology, A. Comparative Physiology 84:127–133.

KUNZ, T. H., O. T. OFTEDAL, S. K. ROBSON, M. B. KRETZMANN, AND C. KIRK. 1995. Changes in milk composition during lactation in three species of insectivorous bats. Journal of Comparative Physiology, B. Biochemical, Systemic, and Environmental Physiology 164:543–551.

Kwiecinski, G. G., L. Krook, and W. A. Wimsatt. 1987. Annual skeletal changes in the little brown bat, *Myotis lucifigus*, with particular reference to pregnancy and lactation. American Journal of Anatomy 178:410–420.

Marier, J. R., and M. Boulet. 1959. Direct analysis of lactose in milk and serum. Journal of Dairy Science 42:1390–1391.

McGee-Lawrence, M. E., H. V. Carey, and S. W. Donahue. 2008. Mammalian hibernation as a model of disuse osteoporosis: the effects of physical inactivity on bone metabolism, structure, and strength. American Journal of Physiology—Regulatory, Integrative and Comparative Physiology 295:R1999–R2014.

McGee-Lawrence, M. E., D. M. Stoll, E. R. Mantila, B. K. Fahrner, H. V. Carey, and S. W. Donahue. 2011. Thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) show microstructural bone loss during hibernation but preserve bone macrostructural geometry and strength. Journal of Experimental Biology 214:1240–1247.

Milligan, L. A., S. V. Gibson, L. E. Williams, and M. L. Power. 2008. The composition of milk from Bolivian squirrel monkeys (*Saimiri boliviensis boliviensis*). American Journal of Primatology 70:35–43.

Murie, J. O. 1992. Predation by badgers on Columbian ground squirrels. Journal of Mammalogy 73:385–394.

Murie, J. O., and D. A. Boag. 1984. The relationship of body weight to overwinter survival in Columbian ground squirrels. Journal of Mammalogy 65:688–690.

Murie, J. O., and F. S. Dobson. 1987. The costs of reproduction in female Columbian ground squirrels. Oecologia 73:1–6.

Murie, J. O., and M. A. Harris. 1982. Annual variation of spring emergence and breeding in Columbian ground squirrels (*Spermophilus columbianus*). Journal of Mammalogy 63:431–439.

Nelson, W. L., A. Kaye, M. Moore, H. H. Williams, and B. L. Herrington. 1951. Milking techniques and the composition of guinea pig milk. Journal of Nutrition 44:585–594.

Nowak, R. M. 1999. Walker's mammals of the world. 6th ed. Johns Hopkins University Press, Baltimore, Maryland.

Oftedal, O. T. 1981. Milk, protein and energy intakes of suckling mammalian young: a comparative study. Ph.D. dissertation, Cornell University, Ithaca, New York.

Oftedal, O. T. 1984. Milk composition, milk yield and energy output at peak lactation: a comparative review. Symposia of the Zoological Society of London 51:33–85.

Oftedal, O. T. 2000. Use of maternal reserves as a lactation strategy in large mammals. Proceedings of the Nutrition Society 59:96–106.

Oftedal, O. T., and S. J. Iverson. 1995. Phylogenetic variation in the gross composition of milks. Pp. 749–789 in Handbook of milk composition (R. G. Jensen, ed.). Academic Press, Inc., San Diego, California.

Peaker, M. E. 1977. Comparative aspects of lactation. Academic Press, London, United Kingdom. Vol. 41.

Phillips, J. A. 1984. Environmental influences on reproduction in the golden-mantled ground squirrel. Pp. 108–124 in The biology of ground-dwelling squirrels: annual cycles, behavioral ecology, and sociality (J. O. Murie and G. R. Michener, eds.). University of Nebraska Press, Lincoln.

Power, M. L., O. T. Oftedal, and S. D. Tardif. 2002. Does the milk of callitrichid monkeys differ from that of larger anthropoids? American Journal of Primatology 56:117–127.

Racey, P. A., and A. C. Entwistle. 2000. Life-history and reproductive strategies of bats. Pp. 363–414 in Reproductive biology of bats (E. G. Chrichton and P. H. Krutzsch, eds.). Academic Press, San Diego, California.

Reiss, M. J. 1989. The allometry of growth and reproduction. Cambridge University Press, Cambridge, United Kingdom.

Robbins, C. T., R. S. Podbielancik-Norman, D. L. Wilson, and E. D. Mould. 1981. Growth and nutrient consumption of elk calves compared to other ungulate species. Journal of Wildlife Management 45:172–186.

SAS Institute Inc. 2002. SAS v.9.1.3 for Windows. SAS Institute Inc., Cary, North Carolina.

Schulte-Hostedde, A., J. S. Miller, and G. J. Hickling. 2001. Evaluating body condition in small mammals. Canadian Journal of Zoology 79:1021–1029.

Shaw, W. T. 1925. Breeding and development of the Columbian ground squirrel. Journal of Mammalogy 6:106–113.

Sikes, R. S., W. L. Gannon, and the Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235–253.

Skibiel, A. L., F. S. Dobson, and J. O. Murie. 2009. Maternal influences on reproduction in two populations of Columbian ground squirrels. Ecological Monographs 79:325–341.

Steinberg, B., I. J. Singh, and O. G. Mitchell. 1981. The effects of cold-stress, hibernation, and prolonged inactivity on bone dynamics in the golden hamster, *Mesocricetus auratus*. Journal of Morphology 167:43–51.

Stern, A. A., T. H. Kunz, E. H. Studier, and O. T. Oftedal. 1997. Milk composition and lactational output in the greater spear-nosed bat, *Phyllostomus hastatus*. Journal of Comparative Physiology, B. Biochemical, Systemic, and Environmental Physiology 167:389–398.

Studier, E. H., and T. H. Kunz. 1995. Accretion of nitrogen and minerals in suckling bats, *Myotis velifer* and *Tadarida brasiliensis*. Journal of Mammalogy 76:32–42.

Sugawara, M., T. Nakamura, T. Ohizumi, and Y. Oki. 1990. Composition of milk and electrophoretic analysis of milk casein in herbivorous voles (*Microtus montebelli*). Experimental Animals 39:281–284.

Utz, J. C., S. Nelson, B. J. O'Toole, and F. van Breukelen. 2009. Bone strength is maintained after 8 months of inactivity in hibernating golden-mantled ground squirrels, *Spermophilus lateralis*. Journal of Experimental Biology 212:2746–2752.

Veloso, C., N. J. Place, and G. J. Kenagy. 2003. Milk composition of free-living yellow-pine chipmunks (*Tamias amoenus*): temporal variation during lactation. Comparative Biochemistry and Physiology, A. Molecular & Integrative Physiology 134:387–392.

Yajima, M., T. Kanno, and T. Yajima. 2006. A chemically derived milk substitute that is compatible with mouse milk for artificial rearing of mouse pups. Experimental Animals 55:391–397.

Young, P. J. 1990. Hibernating patterns of free-ranging Columbian ground squirrels. Oecologia 83:504–511.

Submitted 25 March 2012. Accepted 27 June 2012.

Associate Editor was Paul T. Stapp.