

# Is tissue maturation necessary for flight? Changes in body composition during postnatal development in the big brown bat

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**Abstract** Patterns of offspring development reflect the availability of energy and nutrients, limitations on an individual's capacity to use available resources, and tradeoffs between the use of nutrients to support current metabolic demands and tissue growth. To determine if the long period of offspring dependency in bats is associated with the need for an advanced state of tissue maturation prior to flight, we examined body composition during postnatal growth in the big brown bat, *Eptesicus fuscus*. Despite their large size at birth (22% of maternal mass), newborn bats are relatively immature, containing 82% body water in fat-free mass. However, the total body water content of newborn bat pups decreases to near-adult levels in advance of weaning, while

concentrations of total body fat and protein exceed adult values. In contrast to many other mammals, postnatal growth of bat pups was characterized by relatively stable concentrations of calcium and phosphorus, but declining concentrations of magnesium. These levels remained stable or rebounded in late postnatal development. This casts doubt on the hypothesis that low rates of mineral transfer necessitate an extended lactation period in bats. However, our finding of near-adult body composition at weaning is consistent with the hypothesis that extended lactation in bats is necessary for the young to achieve sufficient tissue maturity to undertake the active flight necessary for independent feeding. In this respect, bats differ from most other mammals but resemble birds that must engage in active flight to achieve nutritional independence.

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## Introduction

Patterns of tissue maturation can provide insight into constraints on postnatal development such as availability of energy and nutrients, limitations on an individual's capacity to use available resources, and tradeoffs at the tissue or cellular level (Ricklefs 1979a; Ricklefs et al. 1998). This should be particularly true during periods of maximal growth, when tissue demand for structural materials is high. Both poor nutrition and very high levels of activity can redirect energetic substrates from muscle growth to catabolic activity, reducing rates of tissue development (Ricklefs 1979a; Ricklefs et al. 1998).

In most mammals, nourishment is provided solely by the mother in the form of milk (Clutton-Brock 1991). Mammalian

milk is highly digestible and acts as an immediate buffer to fluctuations in food supply. There is considerable interest in how species-specific milk composition relates to the nutritional needs of the young, as well as to nutritional constraints on the mother (Oftedal 1984, 2000; Oftedal et al. 1993a). The quality of milk to some degree depends on maternal condition. Milk composition and more notably milk yield can vary with nutritional status of the mother (Doreau et al. 1992; Hood et al. 2009; Rasmussen and Warman 1983; Warman and Rasmussen 1983). Thus, changes in tissue and body composition of young during the postnatal growth period may reflect a trade-off between developmental processes, maintenance requirements of developing tissues, and the supply of nutrients available to the mother.

Because of their volant mode of locomotion and prolonged period of dependency, bats are a particularly interesting group in which the transfer of nutrients from mothers to offspring through milk can be studied. Young bats do not become nutritionally independent until they have achieved about 70% of adult mass: nearly twice the average relative mass (37%) of terrestrial species at weaning (Barclay 1994, 1995; Case 1978; Kunz 1987; Kunz and Hood 2000; Kurta and Kunz 1987; Millar 1977). Because aerial insectivorous species typically consume a low-calcium diet, it has been postulated that low-calcium intake by mothers may constrain mineral deposition and thus the relative rates of skeletal maturation in dependent offspring (Barclay 1994, 1995; Studier and Sevick 1992), yet there is little direct evidence to suggest that mineral intake in bats is suboptimal. For example, Booher (2008) found no difference in the length of forearm or body mass of litters born to big brown bats (*Eptesicus fuscus*) fed a diet that mimicked the low-calcium intake of free-ranging bats versus bats supplemented with 10× more calcium. Booher and Hood (2010) further examined the body composition of these young and found no differences between the low- and high-calcium groups. Based on the calcium content of the feces collected from these animals, it was apparent that supplemented animals did not absorb greater amounts of calcium than is typically obtained from their normal insect diet (Booher and Hood 2010).

Alternatively, the long period of dependency in bats may reflect a need for an advanced state of tissue maturation, and particularly maturation of bone, prior to undertaking sustained flight. Survival at independence depends on the ability of juvenile bats to forage effectively. Because the relative strain on the distal elements of the skeleton is greater for flying bats than species using other forms of locomotion (Swartz and Middleton 2008), insectivorous bats that must capture food on the wing may need to approach or achieve tissue maturity before nutritional independence (Papadimitriou et al. 1996; Swartz et al. 1992).

We studied the development of suckling big brown bats (*Eptesicus fuscus*), an obligate, aerial-feeding insectivore (Kurta and Baker 1990). Our goal was to test the hypothesis that the extended period of suckling in bats yields a high level of tissue maturation and mineral deposition prior to nutritional independence from milk. Chemical maturity is achieved when the concentrations of nutrients in the body reach adult values (Moulton 1923). Because chemical maturity reflects the developmental state of the tissues, the chemical composition of the body can be used as an aggregate indicator of tissue maturation. For this investigation, we characterized changes in total body macronutrient and macromineral composition of bat pups from birth until weaning, and compared our results with data on adult females (Hood et al. 2006). We assessed the prediction that prolonged suckling in bats is associated with an advanced level of tissue maturation by comparing our findings with published data on body composition in non-volant mammals and birds at nutritional independence.

## Methods

Big brown bat pups were collected with their mothers from two maternity colonies located in Hollis and Milford, New Hampshire, USA (42°74'N 71°59'W, 42°83'N 71°68'W, respectively) during June and July 1999. All samples were collected during the 5-week suckling period. At weekly intervals, up to ten pups and their mothers were collected from each colony between 0600 and 0900 h. Animals were transported to Boston University where they were maintained in incubators at 30°C (within the zone of thermoneutrality for this species; Stack 1985). Pups were housed with mothers (allowing them to be suckled by their mothers overnight) and then separated the following morning for at least 2 h prior to being killed, ensuring that the young had minimal gastrointestinal contents. Bats were humanely killed by prolonged exposure to gaseous chloroform (Gannon et al. 2007). Body composition analyses and care of the mothers is described elsewhere (Hood et al. 2006). All pups were weighed ( $\pm 0.01$  g), and lengths of forearm ( $\pm 0.1$  mm) and total epiphyseal gap ( $\pm 0.05$  mm; forth right metacarpal-phalangeal epiphysis; Kunz and Anthony 1982) were measured post-mortem. These measurements were used to estimate the age of pups (Burnett and Kunz 1982; Hood et al. 2002). In 4 years of studying big brown bats in Massachusetts and New Hampshire (1996, 1997, 1998 and 1999), we found no evidence that litter size deviated from two (W.R. Hood, personal observation). Estimates of pup age were slightly more accurate when accounting for the relative age of the mother (Hood et al. 2002), but in the present study we did not know the identity of all mothers. Thus, maternal age was not considered as a variable in

estimates of pup age. Carcasses of euthanized bats were frozen at  $-80^{\circ}\text{C}$  until analyses were completed within 1 year. Protocols used in this study were approved by Boston University's Animal Care and Use Committee.

Analysis of body composition was based on a 4-compartment molecular model with the body divided into water, fat, protein, and mineral (Heymsfield et al. 1997; Reynolds and Korine 2009; Speakman 2001). Carbohydrate storage as glycogen was assumed to be minimal ( $<1\%$  of body mass). Detailed descriptions of analytical methods are described by Hood et al. (2006). Briefly, whole carcasses were thawed and homogenized in a Corning laboratory blender. The dry matter content of homogenates was determined by drying samples to a constant mass at  $60^{\circ}\text{C}$  in a forced convection oven. Fat composition was determined based on the mass of fat extracted from dried samples using petroleum ether in a Soxhlet apparatus ( $0.315 \pm 0.036$  g samples). Nitrogen content was determined using a CHNS/O Elemental Analyzer (Perkin Elmer PE2400 Series II, Shelton, Connecticut;  $8.48 \pm 1.80$  mg samples). Nitrogen content was multiplied by the standard conversion factor for animal carcasses of 6.25 to estimate crude protein (Robbins 1993). Samples for mineral analysis ( $98.4 \pm 10.8$  mg) were digested in a microwave-accelerated reaction system (MARS5, CEM Corp., Mathews, North Carolina) in 10 ml concentrated nitric acid. Calcium, magnesium, sodium, and potassium were determined using atomic absorption spectrophotometry (Smith-Hieftje 12, Thermo-Jarrell Ash, Franklin, MA). Phosphorus was analyzed using the AOAC-modified Gomori method (Horwitz 1980). Mineral recoveries of non-fat milk standard reference material (#1549, National Institutes of Standard and Technology, U.S. Department of Commerce, Gaithersburg, MD) were  $\geq 92.3\%$  of guaranteed values (Hood et al. 2006).

Statistical analyses were conducted using SAS (SAS Institute Inc., Cary, NC). All proportional data were arcsine-transformed for analysis. Fat, protein, and mineral composition are expressed in units that reveal functional trends rather than the confounding effects of change in other constituents (Hood et al. 2006), with total body water content expressed relative to fat-free mass (FFM), fat expressed relative to dry mass (DM), and protein, calcium, phosphorus, magnesium, potassium, and sodium expressed relative to fat-free dry mass (FFDM). Although sodium and potassium could be presented as molar concentrations to reflect their electrolytic functions, a substantial proportion of sodium is associated with bone and thus is insoluble (Spray and Widdowson 1950). Because soluble and insoluble sodium and potassium cannot be distinguished, we present these elements relative to fat-free dry mass. All nutrients are expressed as total body content to document patterns of accretion.

Variation in nutrient accretion during postnatal growth was evaluated relative to sex of pups and colony, using multivariate analysis of covariance (MANCOVA, proc GLM; SAS Institute Inc. 1990). Nutrient contents were set as dependent variables, sex and colony as covariates, and body mass of individual pups as the independent variable. This analysis was limited to pups  $>10$  days old because few pups aged  $\leq 10$  days were collected from one of the colonies (Hollis, NH). Body mass, rather than age of pups, was used for all comparisons presented here because body composition is more strongly correlated with body mass than age (Gardner et al. 1964) and exact pup age was not always known. However, comparisons relative to pup age are included in the supplementary materials to facilitate comparison with other studies. The first two and the final 3 weeks of suckling appear to be distinct phases of postnatal development. Low activity but rapid increase in body mass and development of the wings and pelage characterize the first 2 weeks, whereas reduced rates of postnatal growth, movement within the roost, wing flapping, test flights, and the onset of independent foraging occur during the final weeks of dependency (Hood et al. 2002). To account for the possibility that differences in activity were correlated with a change in pattern of nutrient accretion, we examined patterns of change in body composition with both linear and segmented regressions (SAS proc NLIN) and then presented the regression model that best characterized the data. Because the  $F$  and  $P$  values should not be compared between these two regression models directly due to a change in degrees of freedom, we determined the best fit regression by comparing the least square error values between the statistical models. The regression with the lowest least square error was considered to be the best fit model (Ryan and Porth 2007). In addition, we present data that summarize body composition at birth and weaning (Table 1). To facilitate comparison with other studies, all regression equations are based on non-transformed data and we included regression equations for changes in body composition expressed relative to pup age in online Supplement 1.

To provide context to our results, we compiled data on the body composition of mammals and birds at birth/hatching and weaning/fledging (Tables 3, 4; references, sample sizes, ages, and body masses of animals are given in Supplement 2). We converted all values to common units to facilitate comparisons, and we were forced to exclude studies in which there were not enough data given for unit conversion. Because data for several published studies are only presented as graphs (Dunn 1975; Navarro 1992; Spray and Widdowson 1950), we generated numeric values by digitizing these graphs using the programs DigiMATIC or ImageJ (FEB software, Chesterfield, VA; National Institutes of Health, Bethesda, MD). We included the data in Spray and

**Table 1** Body mass, macronutrient mass, and composition of big brown bats (*Eptesicus fuscus*) at birth and weaning

	Birth	Weaning	Gain during postnatal growth relative to uterine development (%)
<i>N</i>	3	5	–
Age (days)	0–1	30–35	–
Mass (g)	3.40 ± 0.14	11.3 ± 0.4	231
% Adult mass	20–23	65–74	–
Macronutrients			
Dry matter (g)	0.66 ± 0.04	3.43 ± 0.10	417
Water (g)	2.74 ± 0.13	7.68 ± 0.20	180
Protein (g)	0.46 ± 0.03	2.29 ± 0.12	396
Fat (g)	0.07 ± 0.01	0.64 ± 0.10	855
Dry matter (% BM)	19.5 ± 1.0	30.1 ± 0.5	–
Water (% BM)	80.5 ± 1.0	69.8 ± 0.3	–
Water (% FFM)	82.1 ± 0.8	73.4 ± 0.5	–
Protein (% BM)	13.6 ± 0.6	20.6 ± 0.6	–
Protein (% FFDM)	77.5 ± 0.6	82.2 ± 0.4	–
Fat (% BM)	1.99 ± 0.32	5.80 ± 0.96	–
Fat (% DM)	10.1 ± 1.3	18.7 ± 2.9	–
Macrominerals			
Calcium (mg)	28.6 ± 2.0	105.9 ± 4.2	270
Phosphorus (mg)	19.4 ± 1.2	79.5 ± 2.7	310
Magnesium (mg)	1.12 ± 0.5	4.88 ± 0.29	336
Potassium (mg)	9.69 ± 0.29	32.0 ± 1.1	230
Sodium (mg)	4.70 ± 0.16	16.3 ± 1.5	247
Calcium (mg/g FFDM)	48.2 ± 3.8	38.1 ± 1.1	–
Phosphorus (mg/g FFDM)	32.7 ± 2.2	28.7 ± 1.3	–
Magnesium (mg/g FFDM)	1.88 ± 0.08	1.73 ± 0.05	–
Potassium (mg/g FFDM)	16.4 ± 0.8	10.5 ± 0.7	–
Sodium (mg/g FFDM)	7.93 ± 0.40	5.84 ± 0.42	–

Nutrient gain during postnatal growth is also presented as cumulative gain relative to gain in utero. Means presented ± standard error  
*BM* body mass; *FFM* fat free mass; *FFDM* fat free dry mass; *DM* dry mass

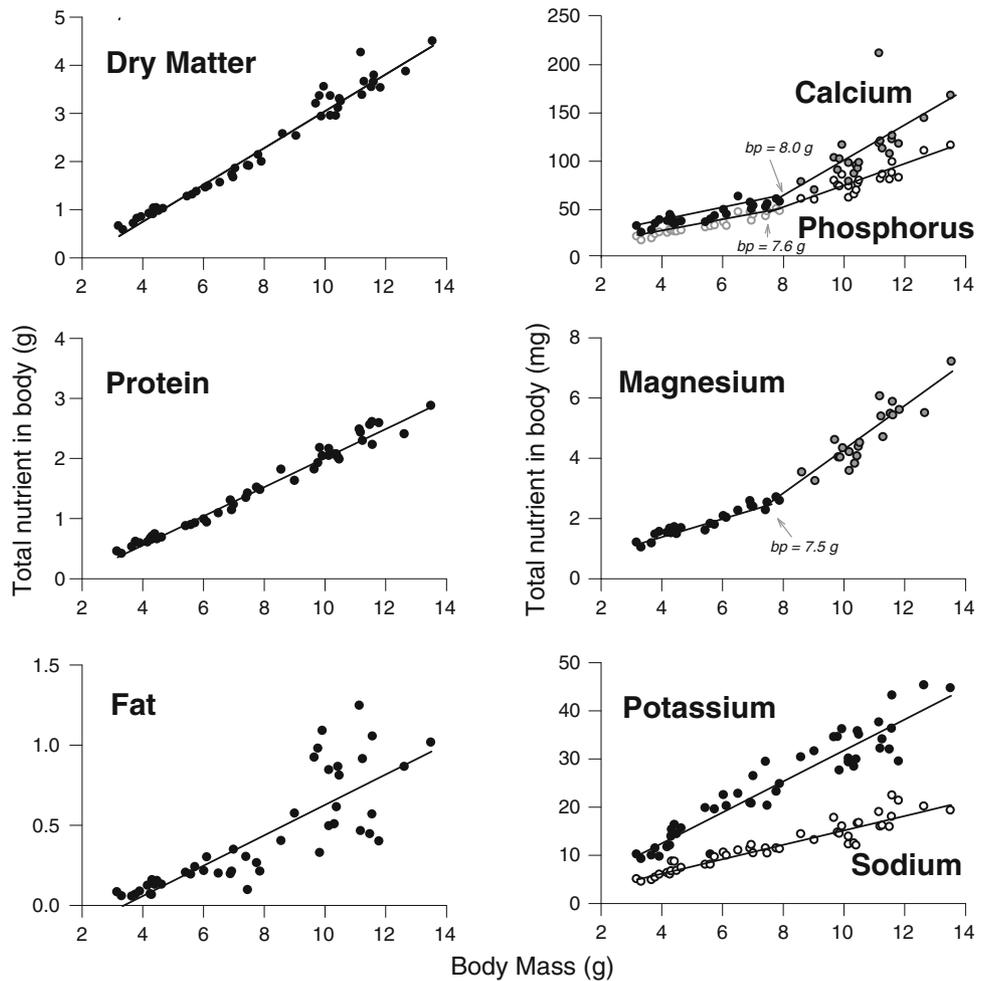
Widdowson (1950) because of the wide variety of species, completeness of mineral data, and its classic role in documenting developmental changes in mammals. Estimates of water content were obtained by difference rather than desiccation and thus may include error. Likewise, protein values in Anderson and Alisauskas (2002) were also determined by difference. Values for body composition from an unpublished study on big brown bats (Stack 1985, Supplement 3) were converted to units comparable to this study. Data on the calcium and sodium composition of cave bat and Brazilian free-tailed bat pups from Studier and Kunz (1995) were also digitized for direct statistical comparison with big brown bats. In the absence of data on water content and fat, the units (mg/g live mass) used by Studier and Kunz (1995) could not be converted to mg/g FFDM; therefore we converted our values to a fresh mass basis for comparison (Fig. 3). The calcium and sodium concentrations of big brown bat, cave bat, and Brazilian free-tailed bat (Studier and Kunz 1995) were compared using ANCOVA (proc GLM; (SAS Institute Inc. 1990). Nutrient contents were

designated as dependent variables, species as independent variables, and pup mass as a covariate.

## Results

Body composition was not significantly influenced by sex of pups whether expressed as nutrient accretion or on a percentage basis (MANCOVA nutrient accretion: Wilk's  $\Lambda = 0.661$ ,  $F_{8,16} = 1.03$ ,  $P > 0.445$ , percent composition: Wilk's  $\Lambda = 0.609$ ,  $F_{8,16} = 1.28$ ,  $P > 0.318$ ). There was also no significant difference in nutrient accretion by colony (MANCOVA: nutrient accretion: Wilk's  $\Lambda = 0.505$ ,  $F_{8,16} = 1.96$ ,  $P > 0.119$ ); however, there was a marginally significant inter-colony difference in the magnesium concentration of pups (MANCOVA: percent composition: Wilk's  $\Lambda = 0.433$ ,  $F_{8,16} = 2.62$ ,  $P < 0.048$ ), with those from the Hollis colony averaging  $1.75 \pm 0.5$  mg/g Mg and those from the Milford colony averaging  $1.62 \pm 0.05$  mg/g Mg. Because this difference is small and no other nutrients

**Fig. 1** Accretion of dry matter, protein, fat, and macrominerals in big brown bat pups during the postnatal growth period expressed as total mass of each nutrient. Regression lines indicate significant changes in composition. Calcium, phosphorus, and magnesium were best explained using a segmented regression. The breakpoint (bp, g body mass) is indicated in each graph. In graphs that present more than one mineral, solid circles correspond with the upper label (calcium or potassium) and the white circles correspond with the lower label (phosphorus or sodium)



differed among colonies, we did not consider colony as a variable in the remaining analyses.

The assayed constituents accounted for  $97.8 \pm 0.1\%$  of total body mass in big brown bat pups. The remaining 2% of the body was primarily attributable to polar lipids (which are not extracted using petroleum ether) and glycogen, although chloride, trace constituents, inaccuracy in the standard protein conversion factor, and minor analytical error may also have contributed.

The macronutrient and mineral composition of big brown bat pups at birth and weaning are summarized in Table 1, and changes in mass of macronutrients and minerals and the relative concentrations of macronutrients and minerals as bat pups increased in body mass are presented in Figs. 1 and 2. Macronutrient and mineral accretion in pups during lactation was typically 200–400% of nutrient accretion during intra-uterine growth, although fat accretion was even greater, 855% (Table 1).

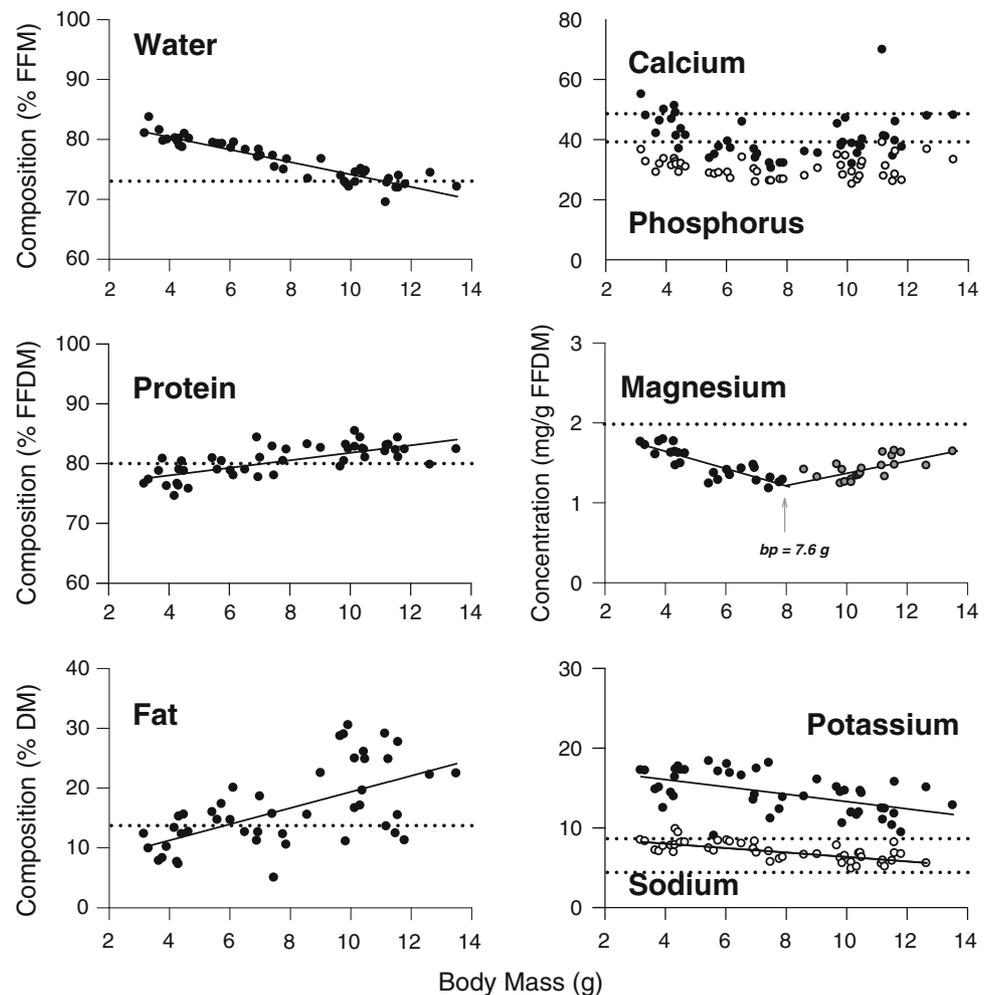
We found significant accretion for all nutrients during postnatal growth (Fig. 1; Table 2). Accretion was linear throughout postnatal development for all nutrients except calcium, phosphorus, and magnesium. Rates of calcium,

phosphorus, and magnesium accretion increased during late postnatal growth (Fig. 1; Table 2). When nutrients were expressed on a proportional basis, linear regression best explained changes in nutrient concentration for all nutrients except magnesium (Table 2). Protein (% FFDM) and fat (%DM) concentrations increased during postnatal growth but concentrations of water (% FFM), phosphorus, and sodium (mg/g FFDM) decreased, while calcium and phosphorus remained unchanged (Fig. 2; Table 2). Magnesium decreased during early postnatal growth, but then recovered prior to weaning (Fig. 2; Table 2).

### Discussion

Chemical and tissue maturation during the postnatal period in big brown bats occurs at an accelerated rate relative to intra-uterine development. Accretion for all macronutrients and macrominerals was two to eight times greater during 5 weeks of suckling relative to 8 weeks in utero. Disproportionate development during postnatal growth would be expected to be pronounced in species that are small at birth,

**Fig. 2** Body composition of big brown bat pups during the post-natal growth period, expressed as percent of total body composition. DM, FFM, and FFDM indicate that values are expressed relative to the dry mass, fat-free mass, and fat-free dry mass of the pups respectively. Regression lines indicate significant changes in composition. Magnesium was best explained using a segmented regression. The breakpoint (bp) for this regression is indicated in the graph. Dotted horizontal lines indicate the average body composition of lactating mothers (Hood et al. 2006). In graphs that present more than one mineral, black circles and the upper dashed line correspond with the upper label (calcium or potassium) and the white circles and lower dashed line correspond with the lower label (phosphorus or sodium)



but bat pups are both large at birth (12–43% of maternal mass) and large at weaning (Kunz et al. 2009; Kunz and Hood 2000; Kurta and Kunz 1987). Thus, young bats experience high nutrient gain in utero and this gain intensifies during suckling as pups approach adult size prior to independence.

#### Body water, protein, and electrolytes

At birth bat pups were at a relatively immature state of development having a body water content of 82.1% of fat-free mass (FFM). By comparison, altricial mammals such as rats, mice, and terrestrial carnivores contain 81–87% water and altricial birds such as passerines contain 88–91% water on a FFM basis; precocial mammals such as seals, ruminants, and horses contain 72–76% water and precocial birds such as ducks and galliforms contain 79–81% water (Table 3). Although the discrepancy in body water content according to developmental state at birth or hatching is well known (Spray and Widdowson 1950; Starck and Ricklefs 1998), the relative chemical immaturity of newborn big

brown bats is surprising, given their large relative size at birth (22% of maternal mass as compared with 0.4–13% in the newborn eutherian mammals and newly hatched birds listed in Table 3).

Bat pups accumulated dry mass linearly from birth until weaning. During this time, the percent of body water decreased while the percent body protein increased, with both water and protein reaching adult levels at or just after mid-dependency. Continued accretion beyond mid-dependency reflects further growth after attaining tissue maturity. Comparable changes in body water have been reported for this and other species of bats during the postnatal period (Reynolds and Kunz 2000; Stack 1985; Supplement 3). We determined the crude protein content of carcasses based on the nitrogen content multiplied by a standard conversion factor, 6.25. Although this conversion factor has been in common use for nearly a century, its validity has not been broadly tested (Rafecas et al. 1994). All studies described in Tables 3 and 4 that directly measure protein use this conversion factor. We recognize that high rates of transcription and translation in developing tissues may entail changes in

**Table 2** Best-fit regressions describing changes in the accretion and concentration of macronutrients and minerals in *Eptesicus fuscus* pups during suckling expressed as a function of body mass

	Break point	Regression	<i>F</i>	<i>df</i>	<i>P</i>	<i>R</i> <sup>2</sup>
Nutrient accretion as a function of mass						
Dry matter (g)	–	(0.306 × mass) – 0.747	1,448	1, 44	<0.001	0.971
Protein (g)	–	(0.242 × mass) – 0.411	2,108	1, 44	<0.001	0.980
Fat (g)	–	(0.095 × mass) – 0.318	94.3	1, 44	<0.001	0.682
Ca (mg)	<8.0	(6.13 × mass) + 10.0	77.9	3, 42	<0.001	NA
	>8.0	(19.0 × mass) – 92.5				
P (mg)	<7.6	(6.09 × mass) – 0.21	21.5	3, 42	<0.001	NA
	>7.6	(11.4 × mass) – 40.8				
Mg (mg)	<7.5	(0.311 × mass) + 0.154	376	3, 42	<0.001	NA
	>7.5	(0.765 × mass) – 3.48				
K (mg)	–	(3.22 × mass) – 0.40	380	1, 44	<0.001	0.896
Na (mg)	–	(1.50 × mass) + 0.19	345	1, 44	<0.001	0.887
Change in composition as a function of mass						
Water (% FFM)	–	(–1.04 × mass) + 84.6	272	1, 44	<0.001	0.860
Protein (%FFDM)	–	(0.63 × mass) + 75.5	45.0	1, 44	<0.001	0.506
Fat (% DM)	–	(1.35 × mass) + 6.02	25.2	1, 44	<0.001	0.364
Ca (mg/g FFDm)	–	NS, <i>x</i> = 40.8 ± 1.1	0.37	1, 44	0.546	0.008
P (mg/g FFDm)	–	NS, <i>x</i> = 30.3 ± 0.5	0.07	1, 44	0.800	0.002
Mg (mg/g FFDm)	<7.6	(–0.11 × mass) + 2.29	21.4	3, 42	<0.001	NA
	>7.6	(0.094 × mass) + 0.75				
K (mg/g FFDm)	–	(–0.46 × mass) + 17.9	18.1	1, 44	<0.001	0.292
Na (mg/g FFDm)	–	(–0.28 × mass) + 9.09	39.4	1, 44	<0.001	0.473

Regression models are linear unless a break point is given. The breakpoint describes the point of intersection between the two regression lines that best characterize the pattern of change in the variable. Means and standard errors are given when change is not significant. Statistical analyses are based on arcsine-transformed values to normalize proportional data but provided regression equations are based on original data to facilitate the use of the equations to calculate body composition

FFM fat free mass; FFDM fat free dry mass; DM dry mass

total nucleic acid: amino acid ratios, but the possible effect on the conversion factor is not clear. Additional research is needed to determine the accuracy of this factor across species and at different developmental stages.

It is well known that sodium is more concentrated in the extracellular solution than within cells (Randall et al. 2001); thus, the postnatal decrease in the concentration of sodium in the body lends support to the assertion that much of the loss of body water is from the extracellular space as tissues mature (Spray and Widdowson 1950). Concentration of potassium was expected to increase with tissue maturation (Spray and Widdowson 1950) rather than decrease as described in the present study. When potassium was expressed relative to body water content (mmol/l of body water), the negative relationship between concentration of total body potassium and body mass was no longer apparent when characterized with linear and segmented regression (*P* > 0.45 and *P* > 0.13, respectively). Findings were similar for sodium when expressed relative to mmol/l body water (linear *P* > 0.76, segmented regression failed to converge on a breakpoint). In some species, up to 50% of the

body’s sodium is stored in bone (Harrison et al. 1936; Spray and Widdowson 1950). Given that sodium and potassium do not run counter to one another, it is possible that bats also deposit a portion of their body’s sodium in bone. Concentration of both of these electrolytes approach adult values by weaning.

Trends in total body water during postnatal development have been described for a limited number of birds and mammals. At maturity, the total body water of birds and mammals is approximately 73% of adult fat-free mass (Widdowson and Dickerson 1964), although this value can be somewhat lower in species with proportionately larger amounts of low-water constituents such as bone, fur, and feathers. In general, total body water at independence in bats was more similar to the body water of fledging birds than weaning mammals (Table 3). Only three species of mammals in Table 3 have lower body water at weaning than bats, and these species include two species of phocid seals and the horse. Some species of seals are born at near-adult concentrations of body water and body water changes little, relative to fat-free mass, during the postnatal period

**Table 3** Percent adult mass, body water, fat, and protein of birds and mammals at birth/hatching (B/H) and weaning/fledging (W/F)

	% Adult mass		Water (%FFM)		Protein (%FFDM)		Fat (%DM)	
	B/H	W/F	B/H	W/F	B/H	W/F	B/H	W/F
Chiroptera								
Big brown bat	22	70	82.1	73.0	77.5	82.3	10.1	14.5
Mammalia								
Rodentia								
House mouse <sup>a,h</sup>	5.9	30	85.1	79.6	85.8	85.8	12.6	20.2
Norway rat <sup>a</sup>	1.5	17	86.4	77.6	80.3	81.2	9.16	21.6
Guinea pig <sup>a</sup>	10	14	78.9	79.3	78.1	81.4	34.7	7.69
Lagomorpha								
Domestic rabbit <sup>a</sup>	3	24	86.7	78.5	81.8	80.1	15.0	23.7
Carnivora								
American mink	1	27	84.8	79.6	87.9	89.4	12.4	40.3
Domestic cat <sup>a</sup>	2	42	82.1	76.2	88.6	82.7	11.3	26.4
Domestic dog	2	–	81.1	–	77.5	–	4.59	–
American black bear	0.4	–	84.5	–	78.0	–	5.64	–
Harp seal	7	28	72.0	71.8	78.7	77.2	9.93	76.0
Hooded seal	13	24	71.7	70.9	81.4	73.3	36.7	73.5
Cetartiodactyla								
Domestic pig	2	11	85.7	79.4	76.0	79.2	7.10	32.6
Domestic sheep	8	19	76.3	76.6	80.4	86.1	10.8	26.3
Dairy cattle	9	40	76.0	73.6	80.4	–	14.8	29.8
Perissodactyla								
Domestic horse	10	73	74.6	69.5	69.2	74.1	9.52	39.1
Aves								
Anseriformes								
King eider <sup>a</sup>	3.1	–	78.9	–	–	–	35.0	–
Galliformes								
Japanese quail <sup>a,c</sup>	5	42.7	81.1	73.2	–	84.8	26.7	30.7
Domestic chicken <sup>c</sup>	2	24	78.7	72.8	84.9	86.6	19.7	21.3
Wild turkey	0.4	2	79.0	75.5	82.4	81.8	29.6	6.44
Pelicaniformes								
Double-crested cormorant <sup>d</sup>	2	88	87.2	68.5	–	–	1.5	17.1
Cape gannet <sup>b</sup>	2	117	77.8	70.9	–	–	33.3	46.4
Charadriiformes								
Common tern <sup>a</sup>	13	–	80.2	–	–	–	8.9	–
Passeriformes								
Common blackbird <sup>f</sup>	4	63	91.1	79.0	71.0	69.4	12.0	8.75
Song thrush <sup>g</sup>	8	79	91.1	78.4	99.4	72.2	12.0	8.33
European starling <sup>a</sup>	7	–	88.0	–	–	–	13.3	–

Units were converted from the original published values to match those in this study where necessary. 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) and fur and feathers were included in the chemical analysis unless otherwise noted. References given in Supplement 2 *FFM* fat free mass; *FFDM* fat free dry mass; *DM* dry mass

<sup>a</sup> Unclear if gut contents removed

<sup>b</sup> Feathers and stomach contents removed

<sup>c</sup> Eviscerated

<sup>d</sup> Adult body mass based on age at 24 weeks, includes gut contents and feathers

<sup>e</sup> Includes gut contents and feathers, Edwards and Denman (1975)

<sup>f</sup> Adult body mass based on Dunning (2008)

<sup>g</sup> Adult body mass based on Hedenstrom and Rosen (2001)

<sup>h</sup> Adult mass based on the average given in National Research Council (1995)

(Ofstedal et al. 1993b). The horse also achieves adult concentrations of body water by weaning, but this follows a long period of reliance on non-milk forage; complete weaning does not occur until approximately 1 year of age. The relationship between body water and the life history of animals is undoubtedly complex. Our observation that near-adult concentrations of body water is common to animals

that fly at independence warrants further investigation, as it suggests that tissue maturity is a prerequisite for flight.

#### Body fat

Bat pups deposit on average eight times more fat during postnatal development than during prenatal development.

**Table 4** Concentration of calcium, phosphorus, magnesium, potassium, and sodium (mg/g fat free dry mass) for birds and mammals at birth/hatching (B/H) and weaning/fledging (W/F)

	Calcium		Phosphorus		Magnesium		Potassium		Sodium	
	B/H	W/F	B/H	W/F	B/H	W/F	B/H	W/F	B/H	W/F
Big brown bat	48.2	36.6	32.7	26.5	1.88	1.73	16.4	10.5	7.93	5.53
Mammalia										
Rodentia										
House mouse	22.8	29.4	23.0	23.8	2.25	1.59	18.4	13.8	15.2	7.55
Norway rat	24.7	38.8	23.7	27.8	1.80	2.24	15.8	15.8	18.0	8.55
Guinea pig	53.5	51.1	35.1	30.2	1.96	1.99	14.2	12.4	8.19	8.96
Lagomorpha										
Domestic rabbit	36.4	–	26.7	30.5	1.76	2.04	16.3	14.6	18.5	8.27
Carnivora										
Domestic cat	38.1	42.3	25.6	26.5	1.49	1.74	13.0	14.1	13.7	7.72
Cetartiodactyla										
Domestic pig	69.0	49.1	39.0	31.1	2.05	1.97	15.0	15.7	16.9	9.26
Dairy cattle	60.9	59.5	34.8	33.8	2.39	–	9.13	–	10.4	–
Perissodactyla										
Domestic horse	–	69.3	–	33.4	–	1.70	–	7.50	–	6.80
Aves										
Passeriformes										
C. blackbird	7.96	24.4	20.46	18.63	1.48	1.33	14.3	10.1	30.7	7.19
Song thrush	6.82	20.9	18.86	16.73	1.32	1.36	20.2	9.82	25.2	7.55

As necessary, units were converted from the original published values. 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) and fur and feathers were included in the chemical analysis unless otherwise noted. References given in Supplement 2; see Table 3 footnotes for comments on sample preparation

Most of this fat appears to be gained during late postnatal growth, although individual variation in fat deposition is extremely high. Fat deposition occurs when dietary intake of energy exceeds requirements for metabolism and lean mass gains. Milk energy output of mothers is highest during late lactation (Hood 2001), coinciding with peak demand of the pups as they become larger, more agile, and begin to fly. High individual variation in fat deposition by bat pups likely reflects a combination of variation in milk energy intake and variation in energy expenditure of pups, and thus the extent to which energy intake exceeds growth and maintenance demands.

A >800% gain in body fat during postnatal development is as much a reflection of low fat deposition in utero as it is the large gain in body mass during the postnatal period. The percentage of fat in the body at weaning was lower than in most mammalian species and some birds. In addition, the fat content in big brown bat pups at weaning in this study was lower than values reported for this species by Stack (1985), indicating that fat deposition may vary among years and that the differing methods of lipid extraction (ether extraction vs. ether-alcohol extraction) may produce differences in recovered fat (see Hood et al. 2006 for a discussion of methodological differences). Interestingly, in our study

the body fat content of many pups exceeded that of their mothers at mid dependency. A high concentration of body fat relative to adults at weaning may function as an ‘insurance policy’ for suckling mammals, increasing the probability of survival as weaned young learn to forage under variable environmental conditions (Muelbert and Bowen 1993; Muelbert et al. 2003). Insectivorous bats do not have a particularly abrupt weaning period (Kunz and Hood 2000), but their food resources (mostly flying insects) may be subject to periods of low availability due to rainfall and low temperatures (Taylor 1963; Williams 1961). Thus, inter-year variation in fat reserves might reflect inter-year variation in food availability to mothers and could be important for the survival of pups if they face temporary food shortages when they are learning to fly and forage.

#### Body calcium, phosphorus, and magnesium

While total body calcium, phosphorus, and magnesium increased throughout the postnatal growth period, concentrations of calcium and phosphorus remained constant while the concentration of magnesium decreased and then subsequently rebounded. Concentrations of calcium and phosphorus in big brown bats are near or below adult levels

from birth until weaning. Our comparison of mineral composition of pups with adults in Fig. 2 is based on an average of females at the onset of lactation (Hood et al. 2006). The mineral content of bone differs between males and females in some mammals. For example, bone mineral content is greater in adult females than in male Norway rats (*Rattus norvegicus*), lower in adult females than males in humans (*Homo sapiens*), but does not differ by sex in the rabbit (Lenchik et al. 2004; Spray and Widdowson 1950). The mineral content of adult male big brown bats is unknown. The mineral content of bone in females increases prior to lactation in some species, including the Norway rat and house mouse, *Mus musculus* (Ellinger et al. 1952; Horst et al. 1997; Sharpe et al. 2003; Wysolmerski 2002), and thus it is possible that the onset of lactation value in our comparison is inflated relative to the bone mineral contents of adult males and non-reproductive females.

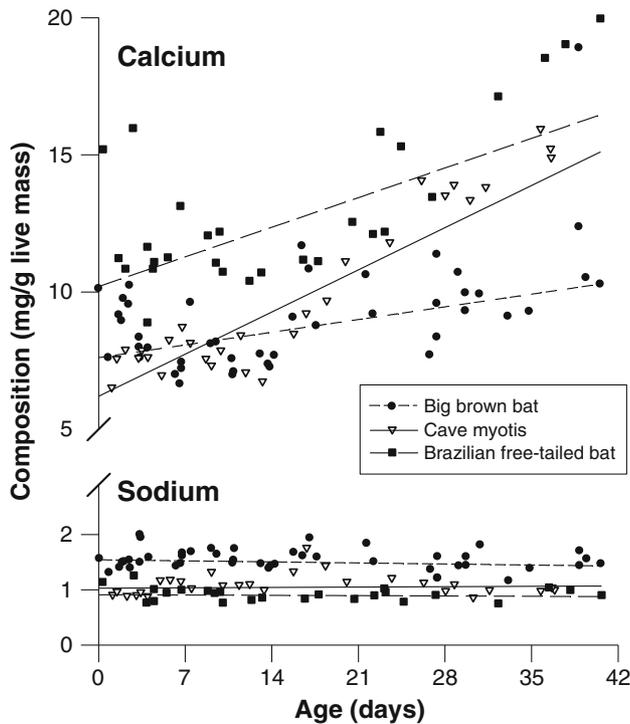
The high concentration of calcium in newborn big brown bat pups appears to reflect a higher level of skeletal mineralization than in many other species (Table 4). Histological data for three additional insectivorous bat species support this result; the level of ossification observed in several skeletal elements of the little brown myotis (*Myotis lucifugus*), southeastern myotis (*Myotis austroriparius*), and Brazilian free-tailed bat (*Tadarida brasiliensis*) are greater at birth than the house mouse and Norway rat at or near weaning (Adams 1992; Hermanson and Wilkins 2008; Patton and Kaufman 1995). Hermanson and Wilkins (2008) described considerable variation in the degree of ossification for different bones in late embryological and early neonatal development in the southeastern myotis and Brazilian free-tailed bat. Thus, a relatively high total body concentration of calcium need not indicate high levels of mineralization for all bones.

Although total body calcium concentration may not have reached adult levels by weaning, it is probable that stable concentrations of total body calcium during development reflect intake of adequate sources of calcium. A decline in the concentration of calcium in bone following birth has been described in several species including domestic cats (*Felis catus*), domestic dogs (*Canis familiaris*), domestic rabbits (*Oryctolagus cuniculus*), Norway rats, and domestic pigs (*Sus scrofa*) (Bauer et al. 1929; Slater and Widdowson 1962), and in the diaphysis of the human femur. Although the decline in bone calcium can be prevented by supplemental calcium, at least in young cats (Slater and Widdowson 1962), this decrease should be considered a normal developmental process that is reversed later in life. The mineral concentration of bone in domestic cats returns to parturition levels by weaning (Dickerson 1962; Spray and Widdowson 1950), whereas human femurs reach mineral concentrations comparable to the time of parturition by age

12 (Dickerson 1962). The early postnatal decline is thought to be associated with the movement of calcium stored in the trabeculae (i.e. cancellous bone) during gestation into the matrix of the growing cortical bone (Widdowson and Dickerson 1964). In addition, a proteinaceous organic matrix is laid down prior to mineralization. During periods of rapid development, the organic matrix can be deposited at a greater rate than mineral and this contributes to a decrease in bone mineral concentration (Teitelbaum 1990). In big brown bats, the pups of mothers supplemented with calcium showed no difference in total body calcium content, compared with the young of unsupplemented mothers (Booher and Hood 2010). Again, these results suggest that intake of calcium is unlikely to affect bone development, at least in big brown bats consuming calcium concentrations typical of free-ranging individuals.

Only one previous study has examined the total body macromineral concentrations of bats during postnatal growth. Studier and Kunz (1995) described the body composition of the cave myotis (*Myotis velifer*) and the Brazilian free-tailed bat, but calcium and sodium were the only elements common to their study and ours (Fig. 3). During early lactation, the calcium content of cave myotis and big brown bat pups were similar, while the calcium content of Brazilian free-tailed bats was significantly higher. During late lactation, however, calcium concentration of big brown bat pups was significantly lower than in the cave myotis and the Brazilian free-tailed bat. The two latter species both produce one offspring, relative to two produced by big brown bats. Thus, it is tempting to conclude that lower total body calcium during late lactation in big brown bat pups relative to cave myotis and Brazilian free-tailed bats may be associated with greater nutritional stress on the big brown bat mothers that are nursing two rather than one young, as in the cave myotis and Brazilian free-tailed bat. However, without data on total body fat and total body water, both of which contribute substantially to variation in total body mass, it is possible that the observed difference among species can be attributed to differences in fat or water, or to inter-laboratory analytical variation. Sodium concentration differed among all three species, but the biological significance of these differences is difficult to interpret.

The relative mass of the skeleton increases with increasing body mass in bats and non-volant mammals, so that the skeletal mass makes a greater contribution to total body mass in larger versus small species (Prange et al. 1979). This difference in the proportion of the body that is skeleton complicates interspecific comparisons. However, it is apparent that concentrations of calcium and phosphorus, but not magnesium, may be greater in big brown bats at birth and weaning than in many larger mammal species (Table 4). Because bone strength is correlated with mineral



**Fig. 3** Calcium and sodium composition of big brown bat pups (this study), cave myotis, and Brazilian free-tailed bat pups (Studier and Kunz 1995) between birth and weaning. Data are expressed in mg/g live mass, as presented in Studier and Kunz (1995). Linear regression lines are presented to emphasize trends for each species

content (Currey 1969), it appears that bats are born with and maintain stronger skeletal elements than other small mammals of comparable body size. With an increase in wing dimensions and no change in calcium or phosphorus concentration within the body, it seems that high accretion rates are important for supporting growth of wing elements that support the mechanical stresses associated with powered flight (Papadimitriou et al. 1996; Swartz and Middleton 2008).

**Conclusions**

The results of this investigation suggest that the prolonged suckling period of bats allows the young to reach or exceed adult body water, protein, and fat concentrations by weaning. Given the large relative mass of muscle compared with other tissues, adult levels of water and protein suggest that muscle maturation is at or near completion at weaning. Concentrations of total body calcium, phosphorus, and magnesium were slightly below adult values at weaning, but relative to other species bats appear to achieve a relatively high degree of bone mineralization by weaning. Thus, findings of this study confirm that the prolonged

lactation period in bats allows pups to reach an advanced state of maturation relative to other mammalian species. It is likely that this advanced state is a prerequisite for the active flight which pups must undertake to become nutritionally independent.

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