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# Milk composition of free-living yellow-pine chipmunks (*Tamias amoenus*): temporal variation during lactation

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## Abstract

Milk is the sole food source of mammals during early postnatal development, and its composition may be modified to meet changing nutritional and energetic demands from birth to weaning. These demands are especially acute in small mammals that breed in highly seasonal environments. We investigated the temporal course of milk composition during lactation in free-living yellow-pine chipmunks, *Tamias amoenus*, a small altricial omnivorous rodent that produces a single annual litter immediately after emerging from hibernation. Over the course of lactation the total energy concentration of milk increased more than two-fold, to approximately 13 kJ ml<sup>-1</sup>. The main component of the milk was lipids, which increased from 10 to 30% of total milk content by wet mass. Proteins increased from approximately 5 to 10%, whereas carbohydrates remained low, at 4–5%. The progressive augmentation of milk energy content during lactation contributes to the increased demands of rapid growth in this altricial species.

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## 1. Introduction

Milk is the exclusive external source of nutrients and energy for infant mammals during early postnatal development. Over the course of neonatal development and maternal lactation the nutritional and energetic requirements of the young change, and the composition of the mother's milk may be modified correspondingly (Green, 1984; Oftedal, 1984a; Oftedal and Iverson, 1995). Energy demand on lactating females often reaches peak lifetime levels (Gittleman and Thompson, 1988; Hammond and Diamond, 1997), and this becomes acute for

mammals of small body size and those that breed in strongly seasonal environments (e.g. Kenagy et al., 1989a).

Analyses of milk composition and energetics of lactation have received attention from a variety of perspectives. These include interspecific variation in body size (Martin, 1984; Gittleman and Thompson, 1988), altricial vs. precocial developmental modes (Derrickson, 1992; Künkele and Trillmich, 1997), temporal pattern of suckling (Ben Shaul, 1962), and phylogenetic affinity (Oftedal, 1984a; Glazier, 1985; Gittleman and Thompson, 1988; Oftedal and Iverson, 1995). Analyses of individual species have examined changes over the course of lactation and development (Baverstock et al., 1976; Green, 1984; Nicholas and Hartmann, 1991; Kunz et al., 1995; Korine and Arad, 1999), variation with regard to litter size (Knight et al., 1986;

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König et al., 1988; Kenagy et al., 1990; Rogowitz and McClure, 1995; Sikes, 1995), maternal diet (Zimmerman et al., 1991; Veloso and Bozinovic, 2000), and maternal age (Knight et al., 1986; Künkele and Kenagy, 1997). We present here a study of lactation in a small altricial omnivorous hibernating rodent species that breeds only once a year in a highly seasonal environment. We are not aware of any previous study of milk composition in a rodent based on field study of a natural population. Apparently nothing is known so far about possible differences between natural milk composition in the field vs. that of captive or laboratory rodents, nor about the possibility that changes in milk composition in the field could be influenced by seasonal changes in food supply or other environmental conditions.

We investigated the temporal course of milk composition during lactation in the yellow-pine chipmunk, *Tamias amoenus*, by studying a natural free-living population. This small (~50 g) altricial species, living in highly seasonal montane forest environments, usually hibernates for approximately 4 months and emerges in late winter or early spring to engage in a single annual bout of reproduction (Broadbooks, 1958; Kenagy and Barnes, 1988). After a gestation of 4 weeks and lactation of five to six weeks, young emerge and become independent near the beginning of summer (Kenagy and Barnes, 1988). The acute nature of energy demand on females for lactation is reflected by the productive output of the growing litter; at birth the litter amounts to approximately 25% of the mother's mass and by weaning it is more than twice the mass of the mother (Kenagy and Barnes, 1988). In light of the altricial pattern of rapid growth and the associated increasing maternal energy demand in these chipmunks, we predicted an increase in the concentration of milk nutrients and energy over the course of lactation from birth to weaning. *Tamias amoenus* is omnivorous and consumes a wide variety of inflorescences, fruits, seeds, corms, tubers, and bulbs of annual and perennial flowering plants, monocots, and fungi, as well as the seeds of coniferous trees; insects and other animal material are a small part of the diet (Broadbooks, 1958; Maser and Maser, 1987).

## 2. Materials and methods

We studied a marked population of yellow-pine chipmunks, under study since 1995, during the

spring breeding season of 1999 at a previously described forested montane site (elevation 670 m) in Chelan County, Washington (Kenagy and Place, 2000; Place and Kenagy, 2000). Animals were captured on a grid of 300 Sherman live traps distributed over an area of 6 ha. We started trapping on 30–31 March, when the first males became active following hibernation, as described in another study (Place et al., 2002). Between 6 April and 23 June we trapped a total of 10 days in April, 16 in May, and 14 in June. We found 24 females in the population during this time, most of which were regularly recaptured. By weighing (Pesola spring balance  $\pm 1$  g) and making repeated external examinations of the females, we were able to judge the course of gestation, closely estimate date of parturition (associated with drop in mass and signs of vaginal disturbance), and track the course of lactation (associated with enlargement of teats and presence of milk).

Milk was collected from females, all of known parturition date, over the course of lactation, ranging from day 2 to day 33. Traps were baited in early morning and checked approximately 2 h later, so that the milk samples were obtained in mid to late morning, upon retrieving chipmunks from the traps. A total of 22 useful samples was obtained from 14 individuals, from five of which repeated samples were obtained at different stages of lactation on two to four occasions. To facilitate milk release and collection, females were injected intramuscularly with 2 IU  $\text{kg}^{-1}$  oxytocin, diluted from a stock of 20 USP  $\text{ml}^{-1}$  (Phoenix Pharmaceutical, St. Joseph, Missouri). Milk was obtained by capillarity, using a Pasteur pipette, from each of the eight teats, following gentle manual expression of the mammary tissue and teat, until no further milk was apparent. Use of the pipette in this manner allowed the samples to be removed without contamination. The sample volumes ranged from 30 to 100  $\mu\text{l}$  and were stored in 0.6 ml plastic tubes at  $-20$  °C until analysis.

Each sample was analyzed for six variables: water content, total solids, protein, carbohydrate, lipid and ash. Water content, total solids and ash were determined gravimetrically (Sartorius Supermicro S3D, to the nearest 0.5  $\mu\text{g}$ ) by weighing a sample of 10  $\mu\text{l}$  and drying it at 100 °C for 24 h in a small aluminum container. Ash content (inorganic matter) was determined using the same sample by burning it twice in a muffle furnace (Nabertherm L3/P) at 560 °C. Protein content

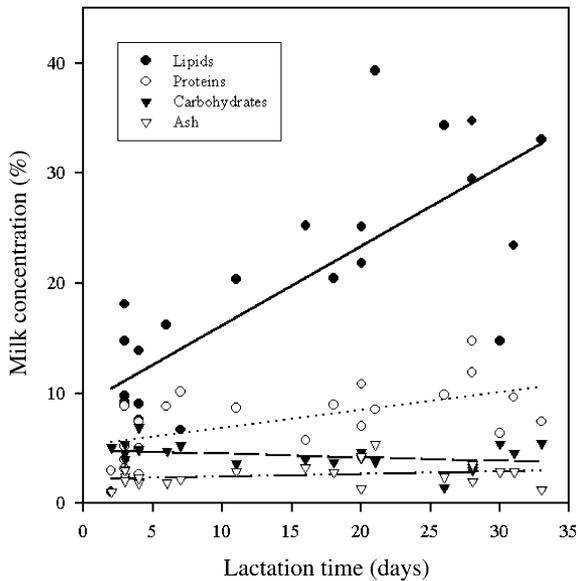


Fig. 1. Concentration (percent of wet mass) of lipids, proteins, carbohydrates, and ash in chipmunk milk over the course of lactation. The lines fitted to the data points are linear regressions, as follows, from top to bottom: (1) lipids,  $y = 0.718x + 9.01$  ( $r^2 = 0.57$ ,  $P < 0.001$ ); (2) protein,  $y = 0.162x + 5.25$  ( $r^2 = 0.36$ ,  $P = 0.003$ ); (3) carbohydrates,  $y = -0.031x + 4.81$  ( $r^2 = 0.099$ ,  $P = 0.15$ ); (4) ash,  $y = 0.024x + 2.19$  ( $r^2 = 0.064$ ,  $P = 0.25$ ).

was determined by analyzing 2  $\mu\text{l}$  of milk by the Coomassie Blue method (Bradford, 1976), using bovine serum albumin as a standard. Carbohydrate was determined on a 2  $\mu\text{l}$  sample by the anthrone procedure using lactose as a standard (Yemm and Willis, 1954). All microvolumetric measurements were made with quantitative grade micropipettes. Lipid content was calculated for each sample by taking the difference between total solids and the sum of carbohydrates, proteins and ash. Finally, energy content of the milk was calculated from the measured milk composition using energetic equivalents of 24.6  $\text{kJ g}^{-1}$  for proteins, 16.5  $\text{kJ g}^{-1}$  for carbohydrates and 38.1  $\text{kJ g}^{-1}$  for lipids (Perrin, 1958). Values of percentage concentration are presented on a wet-mass basis, and total energy content is expressed with respect to volume of whole milk.

We analyzed milk components over the course of lactation by applying linear regression to the arcsine transformed percentage data. Comparisons of body mass were made using Student's *t*-test (Zar, 1996). All values presented are mean  $\pm$  1 S.D.

### 3. Results

Although the first males emerged in late March, our first capture of a female, at the end of the hibernation season, was on 15 April. This followed a lack of female captures on six earlier days of trapping over the previous 16 days. Mating apparently began in mid April, because females first gave birth in mid-May. Mean parturition date was 25 May  $\pm$  6.6 ( $n = 14$ ) with a range of 15 May to 6 June. We obtained a total of 22 milk samples between 18 May and 22 June, ranging from day 2 through day 33 of lactation, based on our individual determinations of parturition dates of the sampled females. This corresponds to a period in which the young remained below ground and were relying exclusively on milk for nutrition (Kenagy and Barnes, 1988).

The temporal distribution of our data falls naturally into categories of early and late lactation, according to the ranges of days 2–11 ( $n = 11$ ) and days 16–33 ( $n = 11$ ), which is apparent from the individual points apparent in the presentation of our regression analyses (Figs. 1 and 2). Milk was relatively dilute in early lactation, with total solids comprising only 20% of milk mass, and by late lactation solids rose to approximately 50%. Lipids were the major constituent of chipmunk milk and

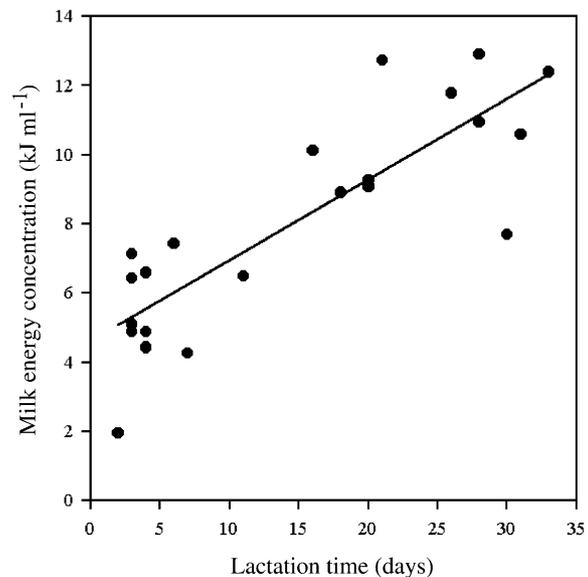


Fig. 2. Total energy concentration of chipmunk milk over the course of lactation. The line is described by the regression:  $y = 0.234x + 4.59$  ( $r^2 = 0.70$ ,  $P < 0.001$ ), where  $y$  is energy concentration of whole milk in  $\text{kJ ml}^{-1}$ .

increased from an initial concentration in early lactation of approximately 10% to greater than 30% in late lactation (Fig. 1). Likewise proteins showed a significant increase over the course of lactation, but generally remained below 10%, even by the end of lactation (Fig. 1). In contrast to lipids and proteins, carbohydrates and ash occupied the smallest fractions of milk composition and neither of these two changed significantly in concentration during lactation (Fig. 1).

Total energy content more than doubled, from approximately 5 kJ ml<sup>-1</sup> in early lactation to an upper range of 12–13 kJ ml<sup>-1</sup> in late lactation (Fig. 2). Because nearly 70% of the nutrient solids are lipid, and because the energy concentration of lipids per unit mass is so much greater than that of proteins and carbohydrates, this means that lipids provide approximately 80% of the energy content of chipmunk milk in late lactation.

It is notable that over the course of lactation the body mass of females did not decline. The mean body mass of females producing the 11 early-lactation samples was 51.6 ± 3.0 g, and that of the 11 from late lactation was 54.6 ± 3.0 g ( $t = -2.35$ ;  $P = 0.03$ ).

#### 4. Discussion

The total energy concentration of the milk of *Tamias amoenus* increased more than two-fold between early and late lactation. This increase was mainly met by the addition of lipids, which tripled in concentration and represented the main mass and energy constituent of chipmunk milk, comprising 80% of milk energy in late lactation. Compared to lipids, proteins were the next most significant component in magnitude and the only other to increase significantly during lactation. The overall pattern we observed in free-living chipmunks is similar to that of many other kinds of mammals in general (Green, 1984; Oftedal, 1984a; Oftedal and Iverson, 1995) and altricial rodents in particular (Baverstock et al., 1976; Nicholas and Hartmann, 1991). Small altricial rodents have the greatest relative growth rates of any mammals (Case, 1978; Reiss, 1989), and the demands of this process on maternal milk energy output increase from a modest initial level following parturition to one of great intensity by peak lactation. In addition to the increase in concentration of energy in milk observed in the present study, the total volume of milk production was thus also

increasing. The mass of an average litter of five *T. amoenus* at the time of emergence onto the surface and weaning is more than twice the mass of the mother (Kenagy and Barnes, 1988). From an ecological perspective the montane forest ecosystem of these rodents shows an increase in abundance and diversity of the food supply, along with warming of environmental temperatures, over the course of the lactation period of these chipmunks (Kenagy et al., 1989a). The season of our study was one of average or above-average favorability of conditions for successful reproduction, and the females were all in good general condition. The maternal diet during lactation probably consisted in large part of the reproductive parts of annual and perennial plants that were growing by mid to late spring (Broadbooks, 1958; Maser and Maser, 1987) and thus should have contained a balance of nutrients, including lipids.

Much like the milk of rodents, that of bats shows an increase in total energy content, largely associated with fat, over the course of lactation (Kunz et al., 1995; Stern et al., 1997; Korine and Arad, 1999). This pattern holds for a variety of insectivorous, frugivorous, and omnivorous bat species. However, as small mammals that fly, bats generally differ in some key life history features from the rodents, having instead smaller litters and slower growth rates. Despite these differences, but likely due to others associated with the costly energy expenditures of flight in bats, lactating females in both these mammalian orders experience high energy demands that increase as lactation progresses; and both groups match these demands by increasing energy content of milk during lactation.

The pattern of growth and development in young of precocial mammal species has been proposed to place less demand on milk energy production than in altricial species, due to the ability of precocial young to obtain their own solid food at an early age (Martin, 1984). However, a study of two precocial rodent species demonstrated relatively high milk energy content and thus did not appear to support this hypothesis (Derrickson et al., 1996). However, precocial young of some species, for example, caviomorph rodents such as the guinea pig (*Cavia porcellus*), are born with a high content of body fat (Widdowson and McCance, 1955), which is directly available metabolic fuel for the young at the beginning of lactation and thus should decrease the demand for maternal

energy via milk. Another precocial caviomorph rodent, the degu, *Octodon degus* maintains a low level of total energy concentration throughout early, mid, and late lactation (Veloso, 1997). The low energy concentration of degu milk is similar to that of chipmunk milk in early lactation, and thus the large increase of milk energy concentration by *T. amoenus* during lactation illustrates a contrast of lactation strategy in altricial rodent species (see also Baverstock et al., 1976; Nicholas and Hartmann, 1991) with that of a precocial rodent. Although two other precocial caviomorph rodents, *Cavia porcellus* (Ofstedal, 1984b) and *Kerodon rupestris* (Derrickson et al., 1996), also show no conspicuous changes of total milk solids content during lactation, these two species have a relatively high level of total solids, in contrast to the low level in degus (Veloso, 1997). Many other factors that vary among precocial rodents, such as diet and ontogeny of feeding and digestion, litter size and mass, as well as body fat of young at birth and weaning, probably account for the further variation among caviomorph species in their patterns of milk energy content.

It is clear from the comparative study of milk composition and energetics (Ofstedal, 1984a; Ofstedal and Iverson, 1995) that lipids are the most important constituent of energy provision in milk. For small mammals such as the chipmunks we have studied here, the large amounts of fat in the milk must all essentially be derived from the daily food intake of the mother. When the balance of energy intake, storage, and expenditure is analyzed in altricial rodents during lactation, it is apparent that no significant amount of the great demand for energy can be derived on a daily basis from maternal fat reserves (e.g. Kenagy et al., 1989a,b). In our study of chipmunks the lack of decline in body mass of mothers from early to late lactation is consistent with the conclusion that female body fat reserves do not make a significant contribution to the fat content of the milk over the course of lactation.

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