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Large litter size increases maternal energy intake but has no effect on UCP1 content and serum-leptin concentrations in lactating Brandt's voles (*Lasiopodomys brandtii*)

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Abstract Lactation is the most energetically demanding period in the female mammal's life. We measured maternal energy intake, uncoupling protein 1 (UCP1) content in brown adipose tissue (BAT), serum-leptin concentration, and litter growth in lactating Brandt's voles (*Lasiopodomys brandtii*) with different litter sizes. Litter mass was positively related to litter size but there was no difference in pup mass at birth. Maternal gross energy intake at peak lactation was positively correlated with litter size and litter mass. Maternal resting metabolic rate (RMR) was positively correlated with litter mass, but not with litter size. No significant differences were detected in body-fat mass, serum-leptin concentration, or UCP1 in lactating voles with different litter sizes. Serum-leptin concentration was negatively correlated with energy intake during lactation. Our data did not support the hypothesis that there is a trade-off in energy allocation between maternal maintenance and offspring growth in lactating Brandt's voles, but support the idea that if the mothers with ten pups should have less energy available for their maintenance than mothers raising fewer pups. Also, leptin is probably not the only factor that induces the high energy intake in mothers with large litter sizes, although it was involved in the regulation of energy intake during lactation.

Keywords Energetic cost · Litter size · Serum-leptin concentration · Uncoupling protein 1 (UCP1)

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Introduction

Lactation is the most energetically demanding period in the life of female mammals and the energy demands of reproduction vary with litter size (Innes and Millar 1981; Mattingly and McClure 1982; Speakman 2007a, b) because trade-offs between number and size of offspring are minimal (Millar 1977). The high energetic cost to mothers with large litter size is mostly met by increasing food intake (Rauw et al. 2002; Liu et al. 2003), and allocating relatively more energy to the offspring (Rauw et al. 2002). However, studies in some species showed significant positive relationships between litter size and energy intake of the mother, but a negative relationship between litter size and offspring growth (Cameron 1973; Myers and Master 1983; Kaufman and Kaufman 1987; Kam and Degen 1994; Mappes et al. 1995; Risch et al. 1995; Speakman 2007a, b). Therefore, the relationship between litter size and maternal maintenance and offspring growth is debatable.

Leptin, the obesity (ob) gene product (Zhang et al. 1994), has been proposed as an adipose-related satiety signal that reduces food intake (Woodside et al. 2000; Mistry and Romsos 2002; Stocker et al. 2004, 2007) and enhances energy expenditure, leading to reduced fat storage and lower body mass (Friedman and Halaas 1998; Ahima and Flier 2000; Schwartz et al. 2000). Conversely, low leptin concentrations after fasting or during lactation are associated

with increased food intake and decreased energy expenditure (Flier 1998; Brogan et al. 1999; Denis et al. 2003). The decrease in serum leptin during lactation seems to be related to litter size in rats, as two pups did not get any suppression in leptin concentration, whereas eight pups did (Brogan et al. 1999), and litter removal increased serum-leptin concentration (Brogan et al. 1999; Denis et al. 2003). Whether leptin is involved in the regulation of food intake in lactating females of other species such as Brandt's voles (*Lasiopodomys brandtii*) with different litter sizes is not clear.

Brandt's voles are non-hibernating herbivores that inhabit mainly the grasslands of Inner Mongolia of China, Mongolia, and the Baikal region of Russia. Liu et al. (2003) showed that metabolizable energy intake at peak lactation in Brandt's voles was increased significantly and correlated with litter size, and the total energy content of the litter was also correlated with litter size. Uncoupling protein 1 (UCP1) content in brown adipose tissue (BAT), which is indicative of nonshivering thermogenesis (NST), is reduced in lactating voles (Li and Wang 2005a), but increased if lactating voles are exposed to cold (Zhang and Wang 2007). Based on these studies, we questioned whether maternal resting metabolic rate (RMR), UCP1 content and offspring growth were correlated with litter size through changes in energy intake, and whether serum leptin was involved in the regulation of energy intake in lactating Brandt's voles. In this way, the physiological and biochemical changes from molecular to whole-organism levels can be integrated in order to test the hypothesis that there is a trade-off in the allocation of energy between maternal maintenance and offspring growth in Brandt's voles. We predicted that energy intake, RMR, and UCP1 content will decrease with increasing in litter size in lactating Brandt's voles, and serum leptin was involved in the regulation of energy intake and thermogenic activity.

Materials and methods

Animals

All animal use procedures were approved by the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Virgin female Brandt's voles, which were the offspring of voles trapped in Inner Mongolian Grasslands in May 1999 and raised in Institute of Zoology, the Chinese Academy of Sciences, were housed in groups of 3–4 per cage (30 × 15 × 20 cm) on sawdust bedding. They were maintained at an ambient temperature of 23 ± 1°C, under a photoperiod of 12L:12D (with lights on at 0800 hours), and offered commercial rabbit pellets (Beijing KeAo Feed Co.) and water ad libitum. Females, weighing 45–55 g and 90–120 days old, were

moved into individual cages for at least 2 weeks before the experiment. The females were paired with males for 4 days to allow mating and then the males were removed. The day of birth was designated as day 0 of lactation. In order to compare the effects of litter size, we selected lactating voles with four pups ($n = 4$), six pups ($n = 6$), eight pups ($n = 6$), and ten pups ($n = 6$). Non-reproductive (NR) voles ($n = 6$) formed the control group. The pups started to eat solid food from day 15 of lactation, so this day was selected as the endpoint because we wanted to measure only the energy consumed by the mother, and this day is included in the peak lactation (days 11–18 of lactation, Johnson et al. 2001; Liu et al. 2003).

Metabolic trials

Resting metabolic rate (RMR) was measured on day 11 of lactation by using an established closed-circuit respirometer at 30 ± 0.5°C (within their thermal neutral zone) as described previously (Wang et al. 2000; Li and Wang 2005b). Briefly, the metabolic chamber volume was 3.6 L and the temperature inside the chamber was maintained by a water bath. KOH and silica gel were used to absorb carbon dioxide and water, respectively, in the metabolic chamber. The voles were weighed before and after each test. After 60 min stabilization in the chamber, oxygen consumption was recorded for another 60 min at 5 min intervals. Two stable consecutive lowest readings were taken to calculate RMR and corrected to standard temperature and pressure (STP). All metabolic measurements were taken between 0900 and 1700 hours to minimize any effects of circadian rhythms.

Energy intake

Food intake was measured between days 12 and 15 of lactation, as described previously (Liu et al. 2003; Li and Wang 2005b). During the test, voles were housed together with their young in stainless steel-mesh metabolism cages (24 × 24 × 24 cm), in which, food and water were provided ad libitum. Uneaten food and feces were collected after the 3-day test, oven-dried at 60°C and separated manually. The caloric value of food was determined using a Parr1281 oxygen bomb calorimeter (Parr Instrument, USA). Gross energy intake was calculated by the equation: gross energy intake (kJ/day) = dry food intake (g/day) × caloric value (kJ/g) of dry food (Liu et al. 2003).

UCP1 measurement

All subjects were sacrificed at 0900–1100 hours on day 15 of lactation at the end of the experiment. Blood samples were collected by puncture of the posterior vena cava. The

interscapular BAT was carefully dissected, frozen in liquid nitrogen and stored at –80°C.

Total BAT protein (15 μg per lane) was separated in a discontinuous SDS-polyacylamide gel (12.5% running gel and 3% stacking gel) and blotted to a nitrocellulose membrane (Hybond-C, Amersham). Unspecific binding sites were saturated with 5% non-fat dry milk in PBS. UCP1 was detected using a polyclonal rabbit anti-hamster UCP1 (1:5,000) (supplied by Dr. M. Klingenspor, Department of Biology, Philipps University, Marburg, Gemany) as a primary antibody, and peroxidase-conjugated goat anti-rabbit IgG (1:5,000) (Jackson Immuno. Inc., USA) as the secondary antibody. Enhanced chemoluminescence (ECL, Amersham Biosciences, England) was used for detection. UCP1 content was expressed as relative units (RU), as determined from area readings by using Scion Image Software (Scion Corporation) (Li and Wang 2005b; Zhang and Wang 2006).

Serum leptin assays

Serum-leptin concentration was determined by radioimmunoassay (RIA) with the ^{125}I Multi-species Kit (Cat. No. XL-85K, Linco Research Inc.) (Li and Wang 2005b; Zhang and Wang 2006). The lowest concentration of leptin that can be detected by this assay was 1.0 ng/ml when using a 100 μl sample. The inter- and intra-assay variability for leptin RIA were <3.6 and 8.7%, respectively.

Body composition analysis

All subjects were sacrificed by decapitation between 0900 and 1100 hours. Trunk blood was collected and the visceral organs, including heart, lung, liver, kidneys, spleen, uterus, and gastrointestinal tract (stomach, small intestine, cecum, proximal colon, and distal colon) were removed and weighed (±1 mg). The stomach and intestines were rinsed with saline to eliminate the contents, before being dried and weighed. The remaining carcass and all the organs were dried in an oven at 60°C to constant mass, and then weighed again to obtain the dry mass. The difference between the wet and dry carcass mass was the water mass of carcass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus.

Data analysis

Data were analyzed using SPSS software package (SPSS 1998). Prior to all statistical analyses, data were examined for normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. Group differences in gross energy intake, RMR, and organ wet and dry mass were analyzed by a one-way analysis of covariance (ANCOVA) with body mass as the covariate and

followed by the least-significant difference (LSD) post-hoc test. Group differences in other parameters (body mass of the mother and pups, carcass mass, UCP1 content in BAT and serum-leptin concentration) were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey HSD post-hoc tests. Pearson correlation was used to determine the associations between litter size and gross energy intake, RMR, UCP1 content and serum-leptin concentration, and the relationship between RMR and body compositions. Results are presented as mean ± SE, and $P < 0.05$ was considered to be statistically significant.

Results

Body mass of mother and offspring

There were no significant differences in body mass among the groups before mating (Table 1). However, the mothers had higher body mass during lactation, but there were no significant differences in body mass among different litter sizes on days 0 and 15 of lactation (Table 1). The mother's body mass before mating ($r = 0.339$, $P > 0.05$), on day 0 of lactation ($r = 0.174$, $P > 0.05$), and on day 15 of lactation ($r = 0.125$, $P > 0.05$) was not significantly correlated with litter size.

There were no significant differences in pup mass among various litter sizes on day 0 or 15 of lactation (Table 1). Litter mass increased with increase in litter size (Table 1) (one pup from one litter of ten and eight, respectively, died on day 13 or 14 of lactation).

Gross energy intake

The mothers who had ten pups had 27–38% higher energy intake than those who had 4, 6 or 8 pups, and had 153% higher gross energy intake than non-reproductive voles ($F_{4,22} = 6.650$, $P < 0.001$; Fig. 1a). Gross energy intake was positively correlated with litter size (Fig. 1b) and litter mass (Fig. 1c).

Resting metabolic rates

Resting metabolic rates (RMRs) of the lactating voles were 57–74% higher than those of the non-reproductive voles ($F_{4,22} = 9.010$, P < 0.001; Fig. 2a). RMR was positively correlated with litter mass (Fig. 2b), but not with litter size ($r = 0.361$, $P > 0.05$).

Brown adipose tissue (BAT) mass and UCP1 content

There were no significant differences in BAT mass among the groups ($F_{4,22} = 0.639$, $P > 0.05$; Fig. 3a). UCP1 content

Table 1 Effects of litter size on the mass of mother and offspring in Brandt's voles

Parameters	NR	Four pups	Six pups	Eight pups	Ten pups	$F_{4,23}$	P
Sample size	6	4	6	6	6		
Mother body mass (g)							
Before mating	46.6 ± 0.7	47.9 ± 0.6	49.7 ± 1.8	48.6 ± 1.7	52.4 ± 1.6	2.244	NS
On day 0 of lactation	47.7 ± 0.9^b	65.0 ± 3.1^a	64.4 ± 2.2^a	62.5 ± 1.6^a	67.9 ± 2.3^a	16.910	<0.001
On day 15 of lactation	47.2 ± 1.0^b	56.3 ± 2.7^a	56.0 ± 2.4^a	54.9 ± 2.1^{ab}	58.2 ± 1.4^a	5.101	<0.01
Changes during 15 days of lactation		8.7 ± 1.2	8.4 ± 2.1	7.7 ± 1.5	9.7 ± 2.1		NS

Parameters	NR	Four pups	Six pups	Eight pups	Ten pups	$F_{3,18}$	P
Offspring body mass (g)							
On day 0 of lactation							
Pup mass (g)		2.6 ± 0.11	2.4 ± 0.05	2.6 ± 0.06	2.4 ± 0.04	2.218	NS
Litter mass (g)		10.4 ± 0.4^d	14.3 ± 0.3^c	20.4 ± 0.5^b	24.2 ± 0.6^a	141.988	<0.001
On day 15 of lactation							
Pup mass (g)		10.7 ± 1.3	9.1 ± 0.7	8.9 ± 0.6	8.8 ± 0.9	0.919	NS
Litter mass (g)		42.7 ± 5.2^c	54.3 ± 4.0^{bc}	71.1 ± 4.8^{ab}	88.3 ± 8.7^a	9.820	<0.001

Data are mean $\pm$ SE. Values within rows with different superscripts are significantly different from each other ($P < 0.05$), determined by a one-way ANOVA and Tukey HSD post-hoc tests. *NR* non-reproductive

in BAT, in the lactating voles with litter sizes of 6, 8, and 10 was significantly lower than that in the non-reproductive voles ($F_{4,23} = 9.269$, $P < 0.001$; Fig. 3b), but UCP1 content was not correlated with litter size ($r = -0.331$, $P > 0.05$) or litter mass ($r = -0.261$, $P > 0.05$) in the lactating voles.

Body-fat mass and serum-leptin concentration

The lactating voles decreased body-fat mass by 33–47%, relative to non-reproductive voles ($F_{4,23} = 7.532$, $P < 0.001$; Fig. 4). Serum-leptin concentration also decreased by 46–52% in the lactating voles compared to that in the non-reproductive voles ($F_{4,22} = 3.771$, $P = 0.018$; Fig. 4) but was not correlated with litter size ($r = -0.005$, $P > 0.05$) or litter mass ($r = -0.052$, $P > 0.05$). serum-leptin concentration was positively correlated with body-fat mass ($r = 0.650$, $P < 0.001$) and UCP1 content in BAT ($r = 0.669$, $P < 0.001$), but negatively correlated with gross energy intake ($r = -0.606$, $P = 0.001$). However, in the lactating voles, there was no significant correlation between serum-leptin concentration and gross energy intake ($r = 0.039$, $P > 0.05$) or UCP1 content in BAT ($r = 0.212$, $P > 0.05$).

Body composition

There were no significant differences in wet carcass mass or fat-free body mass among groups (Table 2). The lactating voles had higher body-water mass (Table 2), higher masses of liver, small intestine, cecum and distal colon (Table 3) than non-reproductive voles. In the lactating voles, there

were no significant differences in body composition or organ mass among different litter sizes (Tables 2, 3).

RMR was positively correlated with dry masses of liver, stomach, small intestine, cecum and colon. However, when the effects of body mass on RMR and body compositions were removed, residual RMR was correlated with residual small intestine, cecum, proximal colon, and distal colon (Table 4).

Discussion

Brandt's voles increased energy intake and mobilized body-fat reserves during lactation, supporting the notion that small rodents can be described as "income breeders" (Jönsson 1997), that is, they rely mainly on increased energy intake to meet the high energy demands of reproduction (Mattingly and McClure 1982; Millar 1987). The fact that mothers with ten pups increased gross energy intake and allocated more energy to their offspring compared to mothers with smaller litter sizes suggests that lactating Brandt's voles compensate for the high energy demands by increasing energy intake. Further, no changes in serum-leptin concentration with changes in litter size suggest that leptin was not the only factor inducing the high energy intake.

Body mass of the mothers and offspring in relation to litter size

The mother's body mass, a decisive factor during reproduction, was correlated positively with the number and quality
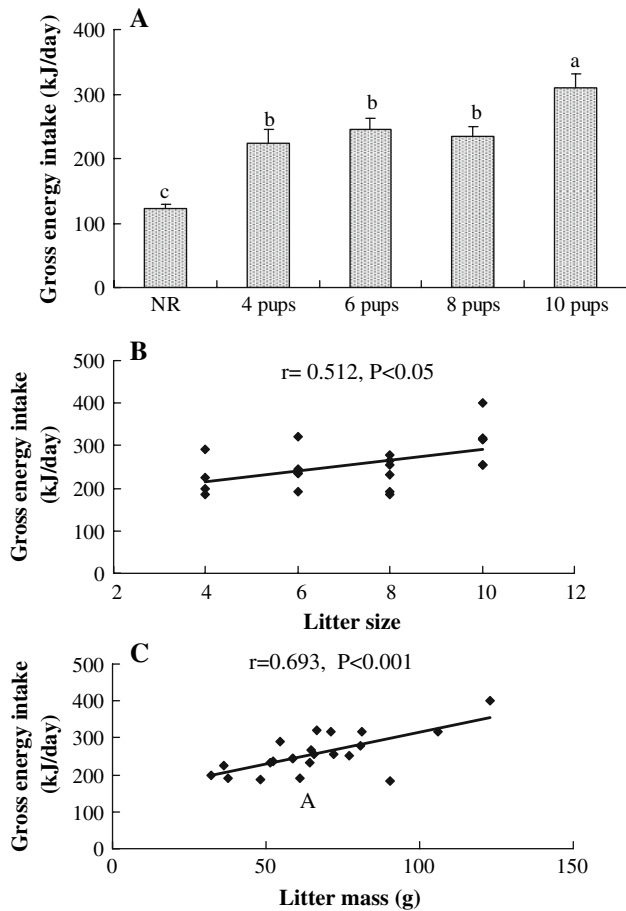
Fig. 1 Effects of litter size on gross energy intake in Brandt's voles. **a** The lactating voles had higher gross energy intake (GEI) than non-reproductive (NR) voles, and the voles with the largest litter size of 10 had the highest GEI. *Error bars* represent mean ± SE. Values with different superscripts are significantly different from each other (*P* < 0.05). **b** Gross energy intake during lactation was positively correlated with litter size. **c** Gross energy intake during lactation was positively correlated with litter mass

Fig. 2 Effects of litter size on resting metabolic rate (RMR) in Brandt's voles. **a** The lactating voles had higher RMR than NR voles, but there were no differences among different litter sizes. *Error bars* represent mean ± SE. Values with different superscripts are significantly different from each other (*P* < 0.05). **b** RMR during lactation was positively correlated with litter mass

Fig. 3 Effects of litter size on **a** brown adipose tissue (BAT) mass and **b** uncoupling protein 1 (UCP1) content in BAT in Brandt's voles. There was no difference among the groups in BAT mass. UCP1 content in the lactating voles with litter size of 6, 8, and 10 was significantly lower than in NR voles. *Error bars* represent mean ± SE. Values with different superscripts are significantly different from each other (*P* < 0.05). **c** Western blotting detection of UCP1 content. The *blots* from the left to right matched those in **b**

of offspring at birth in several rodent species (e.g. Lackey 1976, 1978; McClure 1981; Myers and Master 1983; Kaufman and Kaufman 1987), and mean birth-body-mass and growth rate were negatively correlated with litter size (Cameron 1973; Leon and Woodside 1983; Myers and Master 1983; Kaufman and Kaufman 1987; Mendl 1988; Kam and Degen 1994; Risch et al. 1995). However, in our laboratory study on Brandt's voles, the mother's body mass before mating, on day 0 or 15 of lactation was not correlated with litter size. Further, neither the birth mass nor the pup mass at day 15 of lactation was correlated with litter size. In some rodents, such as white-footed mice (Millar 1978), bank voles (*Clethrionomys glareolus*) (Mappes et al. 1995), and muskrats (*Ondatra zibethicus*) (Boutin et al. 1988), birth masses were not different across different litter sizes. The diverse patterns in different species may reflect different reproductive strategies. Moreover, despite the
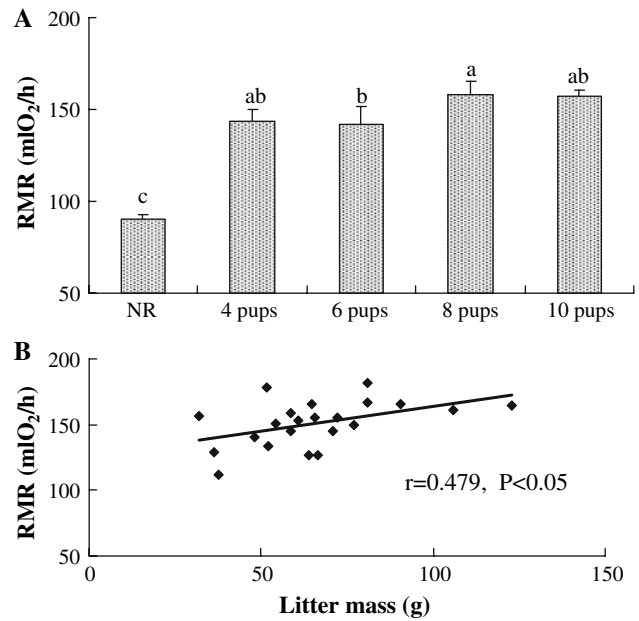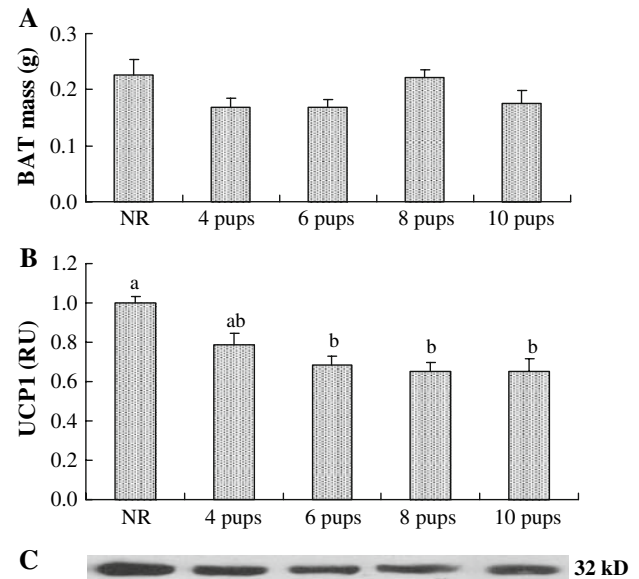
high-correlations among birth mass, mother mass and litter size, some variation in birth mass can be attributed to other factors such as food availability (Millar 1977).
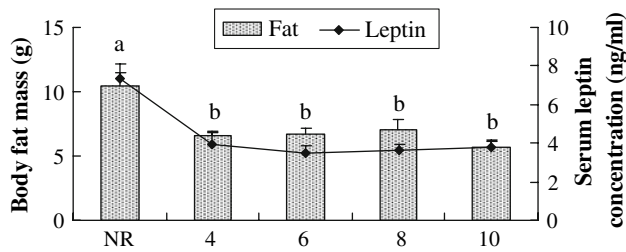
Fig. 4 Effects of litter size on body-fat mass and serum-leptin concentration in Brandt's voles. Both body-fat mass and serum leptin were lower in lactating voles than in NR voles, and there were no differences in the lactating voles with various litter sizes. *Error bars* represent mean ± SE. Values for a specific parameter with different superscripts are significantly different from each other ($P < 0.05$)

Energy intake and RMR in relation to litter size

Lactation is a state characterized by negative energy balance of the lactating female, due to the profound energy drain resulting from milk production (Li et al. 1998). The reproductive costs increase substantially with increase in litter size (Millar 1977, 1978; Sikes 1995; Speakman 2007a, b). The increase in energy demand during lactation is usually met by increased food intake in small mammals (Bartness 1997; Johnstone and Higuchi 2001; Speakman and Król 2005), such as cotton rats (*Sigmodon hispidus*) (Randolph et al. 1977), northern grasshopper mice (*Onychomys leucogaster*) (Sikes 1995), laboratory mice (*Mus musculus*) (Johnson et al. 2001), and Brandt's voles (Liu et al. 2003). Generally, maternal energy intake increased with the increases in litter size and litter mass. Due to the increase in food intake and milk production, RMR during lactation in rodents is also increased (Johnson et al. 2001). RMR was positively correlated with litter mass, suggesting that the energetic cost of maternal maintenance increased with the cost of lactation. Our voles were offered food ad libitum and not subjected to stress conditions, and they were able to compensate for increase in litter size by eating more food. However, different results can be found under natural conditions, for example large-littered species mobi-

lized relatively more energy reserves during lactation and had a lower probability of encountering an energy shortage than the small-littered species (Millar 1987).

The increased masses of digestive organs in lactating Brandt's voles in our study were found in several other rodent species during lactation (Speakman and McQueenie 1996; Hammond 1997; Derting and Austen 1998; Hammond and Kristan 2000; Król et al. 2003; Speakman and Król 2005). These consistent findings suggest plasticity of digestive organs to meet the need for increased energy intake during lactation. Our data also indicate that increased body mass and RMR were consequences of the increases in metabolically active organs.

UCP1 content in BAT in relation to litter size

Besides an increase in food intake, suppression of thermogenic activity is also an adaptive strategy in most rodents during lactation (Isler et al. 1984; Wade et al. 1986; Nizielski et al. 1993). Suppression of thermogenic activity is achieved through the decreases in BAT mass and UCP1 expression (Xiao et al. 2004). In the present study, we found that the decrease in UCP1 content was independent of litter size and litter mass, contrary to the finding of Isler et al. (1984) that thermogenic activity was only suppressed in rat dams nursing large litters. The main reason for this difference could be that Isler et al. (1984) manipulated the litter sizes. Enlargement of litter size over the natural level would be expected to increase reproductive costs (Mappes et al. 1995).

Inhibition of thermogenesis during lactation would save energy for milk production (Trayhurn et al. 1982). Female mice cannot feed their young when temperatures are above the thermoneutral zone, as they are unable to disperse enough heat (Król and Speakman 2003a, b). When the lactating voles were exposed to cold, UCP1 content in BAT increased to the level of cold-exposed non-reproductive voles and helping to maintain a stable body temperature (Zhang and Wang 2007).

Table 2 Effects of litter size on body composition in Brandt's voles on day 15 of lactation

Parameters	NR	Four pups	Six pups	Eight pups	Ten pups	$F_{4,23}$	P
Sample size	6	4	6	6	6		
Wet carcass mass (g)	34.3 ± 0.8	36.5 ± 1.5	35.7 ± 1.1	34.8 ± 1.3	34.9 ± 0.8	0.534	NS
Dry carcass mass (g)	18.0 ± 0.9[a]	15.1 ± 0.3[ab]	15.2 ± 0.6[ab]	15.3 ± 1.0[ab]	13.7 ± 0.5[b]	4.797	<0.01
Body-water mass (g)	16.3 ± 0.3[b]	21.3 ± 1.3[a]	20.5 ± 0.7[a]	19.5 ± 0.7[a]	21.2 ± 0.8[a]	8.482	<0.001
Fat free body mass (g)	7.5 ± 0.2	8.6 ± 0.4	8.5 ± 0.2	8.3 ± 0.5	8.1 ± 0.2	2.149	NS
Body-fat mass (g)	10.5 ± 0.9[a]	6.6 ± 0.3[b]	6.7 ± 0.5[b]	7.0 ± 0.8[b]	5.6 ± 0.5[b]	7.532	<0.001
Body-fat content (%)	30.4 ± 2.3[a]	18.2 ± 1.4[b]	18.7 ± 1.0[b]	20.1 ± 2.1[b]	16.2 ± 1.6[b]	10.148	<0.001

Data are mean ± SE. Values within rows with different superscripts are significantly different from each other ($P < 0.05$), determined by a one-way ANOVA and Tukey HSD post-hoc tests. *NR* non-reproductive

Table 3 Effects of litter size on the wet- and dry-organ mass in Brandt's voles

Parameters	NR	Four pups	Six pups	Eight pups	Ten pups	$F_{4,22}$	P
Wet-organ mass (g)							
Heart	0.237 ± 0.023	0.256 ± 0.025	0.240 ± 0.014	0.224 ± 0.019	0.254 ± 0.029	0.711	NS
Lungs	0.419 ± 0.032	0.322 ± 0.023	0.359 ± 0.024	0.358 ± 0.028	0.451 ± 0.041	2.450	NS
Liver	1.775 ± 0.102[c]	2.471 ± 0.118[bc]	2.640 ± 0.107[ab]	2.577 ± 0.176[ab]	2.984 ± 0.089[a]	5.395	<0.01
Kidney	0.461 ± 0.032	0.544 ± 0.047	0.543 ± 0.025	0.542 ± 0.030	0.612 ± 0.020	0.750	NS
Spleen	0.036 ± 0.003	0.052 ± 0.005	0.049 ± 0.006	0.046 ± 0.005	0.049 ± 0.004	0.252	NS
Uterus	0.098 ± 0.008	0.156 ± 0.028	0.149 ± 0.011	0.128 ± 0.013	0.119 ± 0.019	1.657	NS
Stomach	0.319 ± 0.021	0.416 ± 0.031	0.404 ± 0.013	0.429 ± 0.021	0.459 ± 0.021	2.205	NS
Small intestine	0.581 ± 0.063[b]	1.125 ± 0.063[a]	1.003 ± 0.051[a]	1.047 ± 0.057[a]	1.081 ± 0.093[a]	4.203	<0.05
Cecum	0.388 ± 0.025[b]	0.756 ± 0.084[a]	0.661 ± 0.052[a]	0.704 ± 0.048[a]	0.747 ± 0.029[a]	4.167	<0.05
Proximal colon	0.140 ± 0.007	0.219 ± 0.013	0.243 ± 0.034	0.211 ± 0.018	0.257 ± 0.024	0.865	NS
Distal colon	0.175 ± 0.016[b]	0.405 ± 0.061[a]	0.354 ± 0.023[a]	0.365 ± 0.026[a]	0.441 ± 0.030[a]	3.933	<0.05
Dry-organ mass (g)							
Heart	0.055 ± 0.001	0.065 ± 0.006	0.058 ± 0.002	0.053 ± 0.002	0.059 ± 0.003	1.668	NS
Lungs	0.100 ± 0.005[c]	0.069 ± 0.003[b]	0.079 ± 0.004[ab]	0.074 ± 0.007[ab]	0.094 ± 0.007[a]	4.406	<0.01
Liver	0.539 ± 0.034[b]	0.861 ± 0.081[a]	0.817 ± 0.017[a]	0.783 ± 0.051[a]	0.921 ± 0.051[a]	2.864	<0.05
Kidney	0.113 ± 0.004	0.133 ± 0.009	0.133 ± 0.006	0.133 ± 0.005	0.146 ± 0.006	0.760	NS
Spleen	0.036 ± 0.003	0.052 ± 0.005	0.049 ± 0.006	0.046 ± 0.005	0.049 ± 0.004	1.372	NS
Uterus	0.027 ± 0.003	0.033 ± 0.005	0.032 ± 0.001	0.028 ± 0.003	0.025 ± 0.004	0.887	NS
Stomach	0.075 ± 0.004	0.080 ± 0.016	0.095 ± 0.003	0.101 ± 0.004	0.099 ± 0.003	2.292	NS
Small intestine	0.270 ± 0.018[b]	0.214 ± 0.014[a]	0.196 ± 0.010[a]	0.200 ± 0.009[a]	0.208 ± 0.017[a]	3.369	<0.05
Cecum	0.073 ± 0.005[b]	0.126 ± 0.011[a]	0.111 ± 0.007[a]	0.119 ± 0.007[a]	0.125 ± 0.005[a]	3.826	<0.05
Proximal colon	0.029 ± 0.002	0.042 ± 0.001	0.040 ± 0.002	0.040 ± 0.004	0.047 ± 0.005	1.887	NS
Distal colon	0.048 ± 0.003[b]	0.081 ± 0.011[a]	0.076 ± 0.002[a]	0.076 ± 0.004[a]	0.085 ± 0.004[a]	3.933	<0.05

Data are mean $\pm$ SE. Values within rows with different superscripts are significantly different from each other ($P < 0.05$), determined by a one-way ANCOVA with body mass as a covariate, and least-significant difference (LSD) post-hoc tests. *NR* non-reproductive

Table 4 Correlations between residual RMR and residual dry-organ mass in Brandt's voles

Dry mass	Residual RMR	
	r	P
Residual small intestine	0.434	<0.05
Residual cecum	0.511	<0.01
Residual proximal colon	0.443	<0.05
Residual distal colon	0.481	<0.01

Body-fat content and serum-leptin concentration in relation to litter size

The negative energy balance during lactation is reflected in a decrease in body-fat reserves. Our Brandt's voles, similar to other rodents such as Siberian hamsters (*Phodopus sungorus*) (Schneider and Wade 1987; Bartness 1997) and Syrian hamsters (*Mesocricetus auratus*) (Wade et al. 1986), mobilized body-fat stores during lactation. The maternal fat mass on day 15 of lactation was not related to litter size or litter mass, suggesting that the mothers with larger litters did not mobilize any energy more from body energy reserves. With decreased body fat, lactating voles or rats show decreased serum-leptin concentrations (Brogan et al. 1999; Denis et al. 2003; Xiao et al. 2004; Zhang and Wang 2007). In the present study, serum-leptin concentrations in the lactating voles were not related to litter size or litter mass. However, in rats, the mothers with reduced litter size showed no decrease in the serum leptin (Brogan et al. 1999; Denis et al. 2003). The reduction in litter size would decrease the cost of lactation and may not result in negative energy balance for the mothers.

Our results also show that serum-leptin concentration was negatively correlated with energy intake, but positively correlated with UCP1 content in BAT, suggesting that leptin plays a role in regulating body mass via energy balance. This is partly supported by exogenous leptin administration to lactating rats, which showed that leptin increased UCP1 mRNA and protein production (Xiao et al. 2004), but decreased food intake (Stocker et al. 2004, 2007). When the data were analyzed only from lactating voles, serum leptin

was correlated with neither energy intake nor UCP1 content, suggesting that leptin is not the only factor involved in the regulation of food intake in relation to litter size.

Our data show that reproductive cost increased with increasing litter size and that mothers with large litter sizes increased energy intake to maintain the growth of offspring, these findings do not support the hypothesis that there is a trade-off in energy allocation between maternal maintenance and offspring growth in lactating Brandt's voles, but they do support the idea that the mothers with ten pups should have less energy available for their maintenance than mothers raising fewer pups. Further, our data suggest that leptin is not the only factor in inducing high energy intakes, although it is involved in the regulation of gross energy intake during lactation.

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