J Comp Physiol B (2011) 181:829–839
DOI 10.1007/s00360-011-0560-8

ORIGINAL PAPER

Effects of leptin supplementation to lactating Brandt's voles (*Lasiopodomys brandtii*) on the developmental responses of their offspring to a high-fat diet

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Received: 12 December 2010 / Revised: 4 February 2011 / Accepted: 10 February 2011 / Published online: 3 March 2011
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Abstract Maternal serum leptin concentrations have been suggested as a key factor in programming growth patterns and protecting against adult metabolic disease in human offspring. However, the role of maternal leptin in the development of wild rodent offspring is not clear. We tested the hypothesis that maternal hyperleptinemia in lactating Brandt's voles (*Lasiopodomys brandtii*) can protect their offspring from the risks of high-fat-diet-induced-obesity and insulin resistance. Lactating voles were supplemented with murine leptin (0.64 μg g^{-1} day^{-1}) or phosphate-buffered saline (control) on days10–17 of lactation (peak lactation). At 12 weeks of age, the female and male offspring of the two maternal groups were randomly assigned to two groups each and fed either a high-fat diet (41% of gross energy as fat) or a control diet (14% of gross energy as fat) until the age of 23 weeks. Body mass, food intake, glucose tolerance and resting metabolic rate were determined in the four offspring groups. After animals were sacrificed, organ masses and adipose tissue distribution, and serum leptin and insulin concentrations were measured. Offspring of leptin-treated mothers showed no significant differences in body mass, energy intake or energy expenditure, body composition, glucose tolerance or serum leptin and insulin concentrations from offspring of control mothers. The high-fat diet induced increases in body mass (by 23% in female and 17% in male offspring) and reduced glucose tolerance in both female and male offspring, indicative of the emergence of insulin resistance, even though digestible energy intake of the male offspring decreased on the high-fat diet. These results indicate that maternal hyperleptinemia during peak lactation in Brandt's voles did not protect against diet-induced obesity or glucose intolerance in their offspring.

Keywords Maternal hyperleptinemia · Neonatal programming · Diet-induced obesity · Glucose tolerance · Brandt's vole (*Lasiopodomys brandtii*)

Communicated by I.D. Hume.

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Introduction

The increasing prevalence of obesity across the globe is now widely recognized as a major risk factor for abnormal glucose tolerance, type 2 diabetes, hypertension and cardiovascular disease and as one of today's leading health threats (Stein and Colditz 2004). The reason leading to the weight gain or obesity is due to the positive energy balance, which arises because of decreased energy expenditure, increased energy intake, or a combination of both (Hill 2006). One hypothesis for the prevalence of obesity and associated metabolic syndrome is that our regulatory systems become overwhelmed by the increased access to high-fat and high sucrose palatable foods over the past 30 years (Speakman et al. 2008). There have been many studies on animals that have attempted to characterize the responses of animals exposed to high-fat diets. Most of the available data showed that obesity is induced by high-fat diets, which supports the hypothesis that the amount of fat

in the diet is positively correlated with body fat content (West and York 1998).

More and more epidemiological evidence shows that there is a direct relationship between growth patterns during fetal life and early postnatal life, and an increased risk of adult metabolic disease such as obesity and type 2 diabetes (Barker and Osmond 1986; Ozanne and Constancia 2007; Ravelli et al. 1976). The evidence gives rise to the developmental origins of adult disease hypothesis, which states that environmental factors act in early life can program the risks for adverse health outcomes in adult life (Barker et al. 2002; McMillen and Robinson 2005). This concept is similar to the idea that nutritional and hormonal status during early life could irreversibly impact the development of the organs involved in the control of food intake and metabolism (Djiane and Attig 2008). The mechanisms responsible for this relationship remain poorly understood. However, recent studies indicate that the adipokine-derived hormone leptin may play a critical role in this programming.

Circulating levels of leptin correlate with fat mass, so it can signal body fat level to the brain to control energy homeostasis by regulating the activity of neurons in the arcuate nucleus of the hypothalamus (Friedman and Halaas 1998; Zhang et al. 1994). Lactation is an energetically expensive period for most mammalian species and is characterized by a marked increase in food intake and suppression of thermogenesis (Trayhurn et al. 1982; Wade and Schneider 1992). During this period, maternal leptin levels are suppressed, which has been suggested to drive the induced hyperphagia, thermogenesis and impaired expression of uncoupling protein 1 (UCP1) (Xiao et al. 2004) in brown adipose tissue (BAT). Recent studies have suggested that leptin also plays an important factor for growth and development of the embryo, fetus and infant (Craig et al. 2005; Forhead et al. 2008; Henson and Castracane 2006). During the lactating period, leptin levels play an important role not only in maternal energy balance, but also in the subsequent risk of adult obesity in their offspring. Neonatal rats orally supplied with leptin during the suckling period showed reduced body weight and adiposity as adults (Pico et al. 2007; Sanchez et al, 2008). Leptin administration throughout pregnancy and lactation to both control and malnourished rat dams resulted in an improved metabolic phenotype in the pups when fed a high-fat diet from weaning, and this could be attributed to elevated leptin levels in the milk (Stocker et al. 2004, 2007). To date, the effects of maternal leptin treatment on their offspring has been generated predominantly in laboratory rats and mice, however, the relevance of these results to wild small mammals, unlike lab models which were domesticated for several decades, remains uncertain.

Brandt's voles (*Lasiopodomys brandtii*) are non-hibernating herbivores, mainly inhabiting the Inner Mongolian grasslands of Northern China, Mongolia, and the region of Baikal in Russia. It has been demonstrated that preweaning photoperiod experience can chronically modify their thermogenic capacity (Lu et al. 2007), and the body mass of large litters decreased significantly when their mother lactated at 30°C rather than 21°C (Wu et al. 2009). These findings indicate that environmental conditions experienced by the mother can influence the growth patterns of their offspring. Our laboratory also found that both litter mass and mean pup mass were not affected by the administration of leptin to dams during lactation in Brandt's voles, although their mother's food intake and body mass decreased significantly (Cui et al. 2011). Here, we hypothesized that maternal leptin level during lactation might influence the growth patterns and obesity induced by high-fat diet of the offspring. According to this hypothesis, we predicted that administration of leptin to lactating Brandt's voles would reduce the susceptibility to high-fat diet-induced-obesity in their offspring. Associated parameters, such as energy intake and hormones, fat deposition, and glucose tolerance were also measured.

Materials and methods

Animals and experimental design

All animal procedures were carried out in agreement with the Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences. Brandt's voles used in this study were from our laboratory colony and maintained at 23 ± 1°C on a 16h:8h light: dark cycle (lights on at 04:00). After weaning, the voles were individually housed in plastic cages (30 × 15 × 20 cm) with sawdust as beddings, diets and water were available ad libitum. Commercial rabbit pellets (Beijing KeAo Feed Co.) were used as standard diet, the high-fat diet, purchased from the same company, only differed from standard diet by more lard at the expense of carbohydrate (Table 1).

Table 1 Nutrient composition of the high-fat diet and standard diet (dry-matter basis)

Nutrient	High-fat diet	Standard diet
Gross energy (kJ g^{-1})	21.8	17.1
Total fat (% by mass)	24.1	6.5
Total fat (% by energy)	40.9	14.0
Crude protein (% by mass)	15.6	17.2
Crude fibre (% by mass)	12.5	11.9
Carbohydrates (% by mass)	39.8	56.1
Ash (% by mass)	8.0	8.3

Virgin females were paired with males for a week to allow mating. From day 10 of lactation, mothers with 5–9 pups were selected and divided randomly into a control and leptin administration group. Mean litter sizes of each group were 6.8 and 6.9, which minimized any litter size effects. The control and treatment groups were implanted subcutaneously with Alzet mini-osmotic pumps (Alzet model 2001, capacity 200 μl, release rate 1 μl h^{-1}, Durect Corporation, Cupertino, CA, USA), containing either phosphate-buffered saline (PBS) at pH 7.4 (control group) or recombinant murine leptin (Peprotech, London, UK) (0.64 μg g^{-1} day^{-1}) dissolved in PBS. The pumps delivered the compounds for 7 days. We have shown that this leptin treatment had dose-dependent physiological effects on body mass, food intake and thermogenesis on lactating Brandt's voles (Cui et al. 2011). After 7 days of infusion, the mothers were sacrificed, and the offspring of the same litter were housed in groups until 36 days of age, then they were housed individually until 12 weeks old when random samples were selected (both female and male) and semi-randomized into high-fat diet group and standard diet group until the age of 23 weeks (Fig. 1).

All the animals were sacrificed by CO_2 overdose at age 23 weeks between 09:00h and 11:00 h. The interscapular brown adipose tissue (IBAT) was immediately and carefully dissected, weighed and stored at −80°C until assayed. Blood samples were collected, clotted for 1 h and centrifuged at 4°C for 30 min at 4,000 rpm, sera were then collected and stored at −80°C until assayed.

Table 2 Organ and digestive tract masses in female and male offspring

Parameters	LEP		Control		Statistical summary		
	HFD	SD	HFD	SD	LEP	HFD	LEP×HFD
Organ (♀)	*FLH*	*FLS*	*FCH*	*FCS*			
Heart (g)	0.202 ± 0.007	0.218 ± 0.007	0.223 ± 0.006	0.212 ± 0.006	ns	ns	$P < 0.05$
Liver (g)	1.829 ± 0.097	1.833 ± 0.101	2.165 ± 0.083	1.829 ± 0.089	ns	ns	ns
Spleen (g)	0.038 ± 0.004	0.043 ± 0.005	0.041 ± 0.004	0.036 ± 0.004	ns	ns	ns
Lung (g)	0.450 ± 0.022	0.430 ± 0.033	0.431 ± 0.019	0.457 ± 0.020	ns	ns	ns
Kidneys (g)	0.393 ± 0.014[b]	0.427 ± 0.014[ab]	0.416 ± 0.012[ab]	0.458 ± 0.013[a]	P<0.05	P<0.05	ns
Reproductive organ (g)	0.121 ± 0.013	0.124 ± 0.014	0.129 ± 0.011	0.144 ± 0.012	ns	ns	ns
Organ (♂)	*MLH*	*MLS*	*MCH*	*MCS*			
Heart (g)	0.263 ± 0.009	0.254 ± 0.007	0.250 ± 0.009	0.258 ± 0.008	ns	ns	ns
Liver (g)	2.449 ± 0.106[ab]	2.201 ± 0.088[b]	2.699 ± 0.099[a]	2.327 ± 0.094[ab]	ns	$P < 0.01$	ns
Spleen (g)	0.045 ± 0.005	0.040 ± 0.004	0.047 ± 0.005	0.046 ± 0.004	ns	ns	ns
Lung (g)	0.488 ± 0.031	0.490 ± 0.026	0.454 ± 0.029	0.464 ± 0.027	ns	ns	ns
Kidneys (g)	0.497 ± 0.023[b]	0.573 ± 0.019[ab]	0.479 ± 0.022[b]	0.590 ± 0.021[a]	ns	$P < 0.001$	ns
Testes (g)	0.803 ± 0.065	0.789 ± 0.054	0.828 ± 0.061	0.925 ± 0.058	ns	ns	ns
Epididymis (g)	0.113 ± 0.036	0.148 ± 0.030	0.108 ± 0.034	0.177 ± 0.032	ns	ns	ns
Seminal vesicle (g)	0.577 ± 0.072	0.426 ± 0.060	0.602 ± 0.067	0.479 ± 0.064	ns	ns	ns
Alimentary tract (♀)	*FLH*	*FLS*	*FCH*	*FCS*			
Stomach (g)	0.230 ± 0.014[b]	0.284 ± 0.015[ab]	0.238 ± 0.012[b]	0.291 ± 0.013[a]	ns	$P < 0.001$	ns
Small intestine (g)	0.458 ± 0.045	0.436 ± 0.047	0.585 ± 0.039	0.490 ± 0.041	$P < 0.05$	ns	ns
Ceacum (g)	0.356 ± 0.024[b]	0.431 ± 0.025[ab]	0.390 ± 0.021[ab]	0.467 ± 0.022[a]	ns	$P < 0.01$	ns
Colon (g)	0.321 ± 0.022[ab]	0.356 ± 0.023[ab]	0.315 ± 0.019[b]	0.398 ± 0.021[a]	ns	$P < 0.05$	ns
Alimentary tract (♂)	*MLH*	*MLS*	*MCH*	*MCS*			
Stomach (g)	0.240 ± 0.014[b]	0.306 ± 0.012[a]	0.255 ± 0.013[b]	0.307 ± 0.012[a]	ns	$P < 0.001$	ns
Small intestine (g)	0.602 ± 0.047	0.575 ± 0.039	0.617 ± 0.044	0.636 ± 0.042	ns	ns	ns
Ceacum (g)	0.369 ± 0.025[b]	0.499 ± 0.020[a]	0.369 ± 0.023[b]	0.527 ± 0.022[a]	ns	$P < 0.001$	ns
Colon (g)	0.338 ± 0.019[ab]	0.380 ± 0.016[a]	0.304 ± 0.018[b]	0.386 ± 0.017[a]	ns	$P < 0.01$	ns

Values are least square mean ± SE ($n = 9$–13) from two-way ANCOVA with final body mass as covariate. For each parameter, values with different superscripts differ significantly at $P < 0.05$, determined by a one-way ANCOVA and Bonferroni post hoc tests. Reproductive organ, ovary and uterus

ns not significant, *F* Female, *M* Male, *L* Leptin, *C* Control, *H* High-fat diet, *S* Standard diet

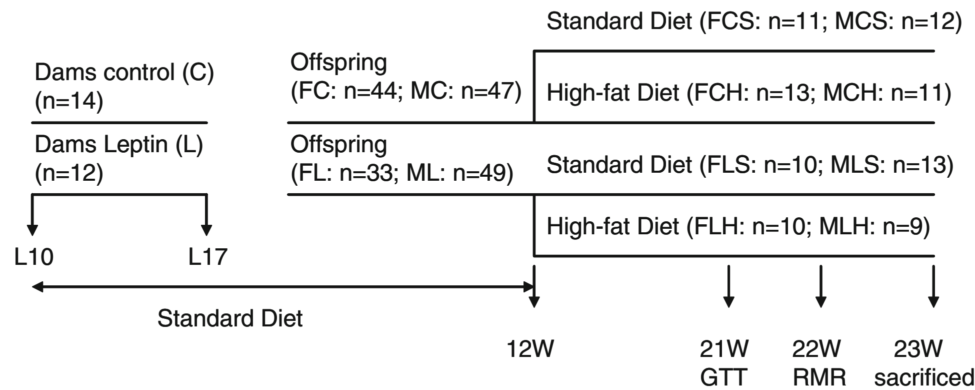

Fig. 1 The design of the experiment. Mothers with 5–9 pups were divided randomly into control (PBS) and leptin administration groups (Mean litter sizes of each group were 6.8 and 6.9) for days 10–17 of lactation, then killed. The offspring of the same nest litter were housed in groups until 36 days old, then they were housed individually until 12 weeks old. 12-week-old offspring of the two maternal groups were stratified for sex then randomized into high-fat diet groups and standard diet groups (a total of 8 experimental groups) until 23 weeks of age, when they were also sacrificed. *F* female, *M* male, *L* maternal leptin treatment, *C* maternal control treatment, *H* high-fat diet, *S* standard (low-fat) diet

Energy balance

Body mass and food intake

Body mass of the offspring (±0.1 g) were weighed (Sartorius, Goettingen, Germany) each week between 3 and 23 weeks of age. Food intake was measured in metabolic cages for three consecutive days of a week during feeding high-fat diet (at 11, 13,15, 19, and 23 weeks of age), after that the food residues and feces were collected, oven-dried at 60°C to constant mass, and separated manually. Dry matter intake (DMI) was calculated from the difference between the food given and food residue. The caloric values of food and feces were determined by bomb calorimetry (Parr Instrument, USA). Digestible energy intake (DEI) was then calculated as follows (Grodzinski and Wunder 1975; Liu et al. 2003; Zhang and Wang 2007):

DEI (kJ day^{-1}) = [Dry matter intake (g day^{-1}) × food gross energy (kJ g^{-1})] − [dry faeces mass (g day^{-1}) × feces gross energy (kJ g^{-1})]

Metabolic trials

Between 07:00h and 20:00h, resting metabolic rate (RMR) of voles was assessed at around 30°C (within their thermal neutral zone) using a FOXBOX O_2 and CO_2 analyzer (Sable systems, NV, USA). Individual voles at the age of 22 weeks were placed in a metabolic chamber (200 × 130 × 85 mm) for 2 h. The flow rate of air (dried with anhydrous $CaSO_4$; W. A. Hammond Drierite Co., USA) was 300–400 ml min^{-1}. Gases leaving the chamber were dried (ND-2; Sable systems, NV, USA) and passed through the oxygen and carbon dioxide analyser at approximately 100 ml min^{-1}. Analyzer outputs were recorded every 6 s. O_2 and CO_2 concentrations in the air were recorded as input concentration for 5 min before and after animal determination. The rate of oxygen consumption was calculated following Hill (1972) and Withers (1977) and RMR was estimated from the stable lowest rate of oxygen consumption over 5 min.

$$\mathrm{RMR} = \frac{\mathrm{FR} \times (\mathrm{FiO_2} - \mathrm{FeO_2}) - \mathrm{FR} \times \mathrm{FeO_2} \times (\mathrm{FeCO_2} - \mathrm{FiCO_2})}{1 - \mathrm{FeO_2}}$$

where FR = flow rate, V = exchange rate for the gas in question (O_2, CO_2), Fi = input fractional concentration, Fe = excurrent fractional concentration.

Body composition and organ mass analysis

After dissection of IBAT, the visceral organs, including heart, thymus, lungs, liver, spleen, kidneys, male testes, epididymis, seminal vesicles or female reproductive organs (including ovary and uterus) were dissected and weighed (±1 mg). The digestive organs (including stomach, small intestine, caecum, and colon) were rinsed with saline to eliminate all the gut contents and weighed (±1 mg). Mesenteric fat pad, female gonadal or male epididymal fat pad, retroperitoneal fat pad and subcutaneous fat pad were also dissected carefully and weighed (±1 mg). The remaining carcass and fat pad were weighed and oven-dried at 60°C to constant mass, and then weighed again to obtain the dry mass. The difference between the wet carcass mass and dry carcass mass was the water mass of carcass. Total body fat extraction was performed with a Soxtec Fat Extraction Systems (Soxtex Avanti 2050, FOSS, Sweden), and then fat free dry carcass mass was

calculated from the difference between dry carcass mass and total body fat.

Serum leptin and insulin level assay

Serum leptin concentrations were measured by radioimmunoassay (RIA) with a ^{125}I multi-species kit (Cat. No. XL-85K, Linco Research Inc. USA) (22, 59). The lower and upper limits of the assay kit were 1 and 50 ng/ml and the intra- and inter-assay variations were <3.6 and 8.7%, respectively. Serum insulin concentrations were measured by radioimmunoassay (RIA) with a ^{125}I human kit (Atom HighTech Co., Ltd., Beijing, CHN). The lower and upper limits of the assay kit were 5 and 160 ng ml^{-1} and the intra- and inter-assay variations were <10 and 15%, respectively. Serum leptin and insulin concentrations were determined in a single RIA and expressed as ng ml^{-1}.

Intra-peritoneal glucose tolerance test

Intra-peritoneal glucose (2 g kg^{-1} body mass) tolerance tests were conducted after fasting overnight. Blood samples were taken by tail veni-puncture between 08:00h and 11:00h for glucose measurements by FreeStyle Mini Blood Meter (Abbott Diabetes Care Inc. Alameda, USA). Immediately before, 15, 30, 60 and 120 min after intra-peritoneal glucose administration, blood glucose was measured (Baur et al. 2006). The linear trapezoidal rule was used for estimation of area under the curve (AUC) (Purves 1992).

Statistical analyses

Data were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. The effects of maternal leptin treatment and feeding the high-fat diet on body mass, and blood glucose tolerance were analyzed by repeated-measures ANOVA and a two-way analysis of variance (ANOVA) followed by Bonferroni post hoc tests. Group differences of dry matter intake and digestible energy intake were analyzed by repeated measures ANCOVA and a two-way analysis of variance (ANCOVA) (leptin and high-fat diet treatment) with body mass as covariate followed by Bonferroni post hoc tests. Group differences in wet organ mass, adipose tissue distribution and RMR were analyzed by a two-way analysis of covariance (ANCOVA) with body mass as covariate followed by Bonferroni post hoc tests. Group differences in other parameters (body compositions, UCP1 content, leptin and insulin concentrations) were analyzed by a two-way ANOVA followed by Bonferroni post hoc tests. Results are presented as mean ± SE, and $P < 0.05$ was considered to be statistically significant.

Results

Offspring body mass

Prior to feeding on the high-fat diet, male voles exhibited greater mass increases than female voles, with no significant difference between leptin and control groups (sex effect, $P < 0.001$; leptin effect, $P = 0.659$; repeated measures ANOVA, Bonferroni correction, Fig. 2a).

When fed the high-fat diet, female offspring increased in body mass and reached maximal levels after 4 weeks (high-fat diet effect, $P < 0.01$; leptin effect, $P = 0.478$; repeated measures ANOVA, Bonferroni correction, Fig. 2b). A similar pattern of increased body mass was detected in male offspring fed the high-fat diet (high-fat diet effect, $P < 0.05$; leptin effect, $P = 0.681$; repeated measures ANOVA, Bonferroni correction, Fig. 2c). Leptin administration had no effect on body mass in either female or male offspring.

Body composition and organ mass

After feeding the high-fat diet for 12 weeks, body mass of female and male voles increased by 9.2 and 9.4 g, respectively, and there were parallel increases in wet carcass mass (Female: $F_{1,40} = 11.248$, $P < 0.01$; Male: $F_{1,41} = 9.219$, $P < 0.01$), dry carcass mass (Female: $F_{1,40} = 13.691$, $P < 0.001$; Male: $F_{1,41} = 7.784$, $P < 0.01$) and fat-free dry carcass mass (Female: $F_{1,40} = 8.905$, $P < 0.01$; Male: $F_{1,41} = 9.546$, $P < 0.01$). Body water mass was not significantly altered by the diet. No effects of maternal leptin treatment on body composition were found in the offspring (Table 3).

Female offspring fed high-fat diet increased total white adipose tissue ($F_{1,39} = 7.528$, $P < 0.01$) by 35%, which resulted from increases in mesenteric ($F_{1,39} = 6.787$, $P < 0.05$), gonadal ($F_{1,39} = 7.386$, $P < 0.05$), and subcutaneous ($F_{1,39} = 5.978$, $P < 0.05$) white adipose tissue. There was no significant increase in fat accumulation in the male offspring due to dietary fat content, except for epididymal fat ($F_{1,40} = 4.219$, $P < 0.05$). No significant differences in the fat depots between the leptin and control groups were found in either female or male offspring, except for greater mesenteric fat in the female offspring on the high-fat diet ($F_{1,39} = 6.716$, $P < 0.05$) (Table 3).

For the mean wet masses of most tissues, there were no significant differences between maternal leptin and control groups except for female kidneys and small intestine. The high-fat diet markedly decreased the mass of the

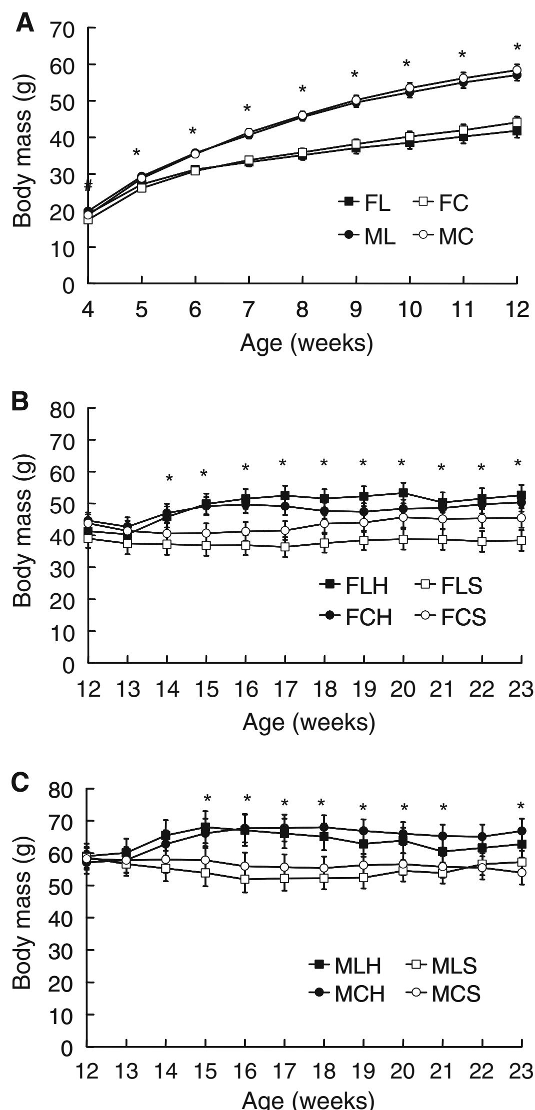

Fig. 2 **a** Effects of maternal leptin treatment and a high-fat diet on body mass of the offspring between 4 and 12 weeks of age on the standard diet. Values are mean ± SE ($n = 33$–49). $^{*}P < 0.05$ *M* versus *F* offspring, $^{\#}P < 0.05$ *L* versus *C* offspring (two-way ANOVA). **b**, **c** The high-fat diet (H) induced mass gain in both the female (**b**) and male offspring (**c**). Values are mean ± SE ($n = 9$–13). $^{*}P < 0.05$ *F* versus *S* offspring (two-way ANOVA). *F* female offspring, *M* male offspring, *L* maternal leptin treatment, *C* maternal control treatment, *H* offspring high-fat diet, *S* offspring standard (low-fat) diet

alimentary tract of both the female and male offspring, including stomach (Female: $F_{1,39} = 13.517$, $P < 0.001$; Male: $F_{1,40} = 19.406$, $P<0.001$), caecum (Female: $F_{1,39} = 9.549$, $P < 0.01$; Male: $F_{1,40} = 37.416$, $P<0.001$) and colon (Female: $F_{1,39} = 6.576$, $P<0.05$; Male: $F_{1,40} = 11.971$, $P < 0.01$), but not the small intestine (Table 2). There were significant increases for the kidneys of both sexes (Female: $F_{1,39} = 7.270$, $P < 0.05$; Male: $F_{1,40} = 17.823$, $P < 0.001$) and for the liver of male offspring ($F_{1,40} = 9.275$, $P < 0.01$) on the high-fat diet.

Energy intake

Female offspring fed the high-fat diet for 4 weeks markedly decreased their dry matter intake (DMI) when compared to standard diet controls, after which they maintained this level of DMI until sacrificed (high-fat diet effect, $P < 0.01$; leptin effect, $P = 0.565$, repeated measures ANCOVA, Bonferroni correction, Fig. 3a). Male offspring fed the high-fat diet showed an even greater decrease in DMI compared to standard diet controls (high-fat diet effect, $P < 0.001$; leptin effect, $P = 0.571$, repeated measures ANOVA, Bonferroni correction, Fig. 3b).

Female offspring fed the high-fat diet showed no significant change in digestible energy intake (DEI) compared with standard diet controls (leptin effect, $P = 0.471$; diet effect, $P = 0.111$; age effect, $P = 0.374$, repeated measures ANOVA, Bonferroni correction, Fig. 3c), except for a transient increase after 2 weeks on the high-fat diet. In contrast, male offspring fed the high-fat diet decreased DEI compared with the standard diet controls after 4 and 8 weeks on the high-fat diet (leptin effect, $P = 0.657$; diet effect, $P < 0.05$, Fig. 3d); mean DEI decreases were 17.7 kJ day^{-1} (16.2%) after 4 weeks and 24.6 kJ day (21.2%) after 8 weeks. This effect disappeared after 12 weeks. There were no significant effects of the maternal leptin treatment on DMI or DEI in either female or male offspring.

Energy metabolism

Resting metabolic rate was unaffected by the high-fat diet or leptin treatment in both female and male offspring (Fig. 4).

Serum leptin and insulin concentrations

The high-fat diet treatment resulted in an approximately fivefold higher concentration of female offspring circulating insulin compared with standard diet controls ($F_{1,40} = 6.059$, $P < 0.05$). No significant differences in leptin or male insulin were detected after feeding the high-fat diet. Maternal leptin treatment had no significant effect on either female or male offspring leptin or insulin concentrations (Fig. 5).

Intraperitoneal glucose tolerance test

After the high-fat diet treatment, blood glucose concentrations were significantly increased in both female (high-fat diet effect, $P < 0.01$; leptin effect, $P = 0.157$, repeated measures ANOVA, Bonferroni correction, Fig. 6a) and male offspring (high-fat diet effect, $P < 0.001$; leptin effect, $P = 0.892$, repeated measures ANOVA, Bonferroni

Table 3 Body composition and adipose tissue distribution in female and male offspring

Parameter	LEP		Control		Statistical summary		
	HFD	SD	HFD	SD	LEP	HFD	LEP×HFD
Body composition (♀)	*FLH*	*FLS*	*FCH*	*FCS*			
Final body mass (g)	52.6 ± 3.2[a]	38.5 ± 3.2[b]	50.3 ± 2.8[ab]	45.5 ± 3.1[ab]	ns	$P < 0.01$	ns
Wet carcass (g)	38.6 ± 2.6[a]	27.0 ± 2.6[b]	36.7 ± 2.3[a]	31.8 ± 2.4[ab]	ns	$P < 0.01$	ns
Dry carcass(g)	21.6 ± 2.1[a]	12.6 ± 2.1[b]	20.4 ± 1.8[a]	14.8 ± 2.0[ab]	ns	$P < 0.001$	ns
Body water(g)	16.9 ± 0.8	14.3 ± 0.8	16.3 ± 0.7	17.0 ± 0.8	ns	ns	ns
Fat free dry carcass(g)	8.2 ± 0.4[a]	6.5 ± 0.4[b]	7.8 ± 0.4[ab]	7.1 ± 0.4[ab]	ns	$P < 0.01$	ns
Body composition(♂)	*MLH*	*MLS*	*MCH*	*MCS*			
Final body mass (g)	62.8 ± 4.2	57.2 ± 3.5	65.8 ± 4.0	53.9 ± 3.6	ns	$P < 0.01$	ns
Wet carcass (g)	46.5 ± 3.2[ab]	40.4 ± 2.6[ab]	47.9 ± 3.0[a]	37.4 ± 2.8[b]	ns	$P < 0.01$	ns
Dry carcass(g)	22.7 ± 2.5	18.2 ± 2.1	23.3 ± 2.4	16.0 ± 2.2	ns	$P < 0.01$	ns
Body water(g)	23.7 ± 1.4	22.2 ± 1.2	24.6 ± 1.3	21.3 ± 1.2	ns	ns	ns
Fat free dry carcass(g)	10.2 ± 0.5[ab]	9.2 ± 0.4[ab]	10.5 ± 0.5[a]	8.5 ± 0.4[b]	ns	$P < 0.01$	ns
Adipose tissue (♀)	*FLH*	*FLS*	*FCH*	*FCS*			
Mesenteric fat (g)	0.524 ± 0.048[a]	0.409 ± 0.050[ab]	0.421 ± 0.041[ab]	0.280 ± 0.044[b]	$P < 0.05$	$P < 0.05$	ns
Gonadal fat (g)	1.141 ± 0.171[ab]	0.883 ± 0.178[ab]	1.329 ± 0.147[a]	0.634 ± 0.157[b]	ns	$P < 0.05$	ns
Retroperitoneal fat (g)	1.014 ± 0.135	1.008 ± 0.140	1.127 ± 0.116	0.684 ± 0.124	ns	ns	ns
Subcutaneous fat (g)	3.728 ± 0.346	3.149 ± 0.361	3.718 ± 0.298	2.560 ± 0.319	ns	$P < 0.05$	ns
Total WAT (mg)	6.407 ± 0.603[ab]	5.448 ± 0.629[ab]	6.595 ± 0.519[a]	4.158 ± 0.556[b]	ns	$P < 0.01$	ns
Adipose tissue (♂)	*MLH*	*MLS*	*MCH*	*MCS*			
Mesenteric fat (g)	0.362 ± 0.031	0.329 ± 0.026	0.274 ± 0.029	0.340 ± 0.028	ns	ns	ns
Epididymal fat (g)	1.040 ± 00.142[ab]	0.966 ± 0.118[b]	1.476 ± 0.133[a]	0.989 ± 0.126[ab]	ns	$P < 0.05$	ns
Retroperitoneal fat (g)	0.960 ± 0.184	0.847 ± 0.153	1.326 ± 0.173	0.862 ± 0.164	ns	ns	ns
Subcutaneous fat (g)	3.572 ± 0.564	2.536 ± 0.470	3.954 ± 0.528	3.102 ± 0.501	ns	ns	ns
Total WAT (mg)	5.934 ± 0.854	5.677 ± 0.711	7.030 ± 0.800	5.293 ± 0.759	ns	ns	ns

Values of body composition are mean ± SE ($n = 9$–13) (two-way ANOVA).Values of adipose tissue are least square mean ± SE ($n = 9$–13) from two-way ANCOVA with final body mass as covariate. For each parameter, values with different superscripts differ significantly at $P < 0.05$, determined by a one-way ANOVA or one-way ANCOVA and Bonferroni post hoc tests. Reproductive organ, ovary and uterus

ns not significant, *F* Female, *M* Male, *L* Leptin, *C* Control, *H* High-fat diet, *S* Standard diet

correction, Fig. 6c). Glucose area under the curve (AUC) from 0-120 min was also higher in high-fat diet treated female ($F_{1,28} = 8.081$, $P < 0.01$, by 46%) and male ($F_{1,28} = 30.906$, $P < 0.001$, by 48%) offspring than in their respective controls. No significant differences were found in relation to maternal leptin treatment in female ($F_{1,28} = 1.526$, $P = 0.227$) or male offspring ($F_{1,28} = 0.150$, $P = 0.701$) (Fig. 6b, d).

Discussion

Like other small mammal species such as mice (Johnson et al. 2001), rats (Villarroya et al. 1986), cotton rats (Randolph et al. 1977) and deer mice (Perrigo 1987), Brandt's voles meet their high energetic costs of lactation by increasing food intake (Liu et al. 2003), and also by decreasing thermogenic capacity in BAT (Li and Wang 2005). Serum leptin appears to be involved in energy intake regulation and thermoregulation (Zhang and Wang 2007), and Cui et al. (2011) has demonstrated that maternal leptin administration during lactation in Brandt's voles can reverse these metabolic consequences.

Results from the present study demonstrate that maternal leptin treatment during peak lactation (days 10–17) in voles did not affect body mass gain, energy intake, RMR, adaptive thermogenesis in BAT, most of the inner organs and fat pad mass, serum insulin and leptin concentrations and glucose tolerance in both female and male offspring. Moreover, maternal hyperleptinemia had no significant effect on susceptibility of their offspring to high-fat diet-induced obesity. Vickers et al. (2005) also found that neonatal leptin treatment during early lactation (days 3–13) had no demonstrable effects on the adult offspring of normally fed female rats. Studies on developmental programming have demonstrated that leptin treatment to both the mother and neonates prevents the induction of the obese phenotype in later life. However, there are reports of

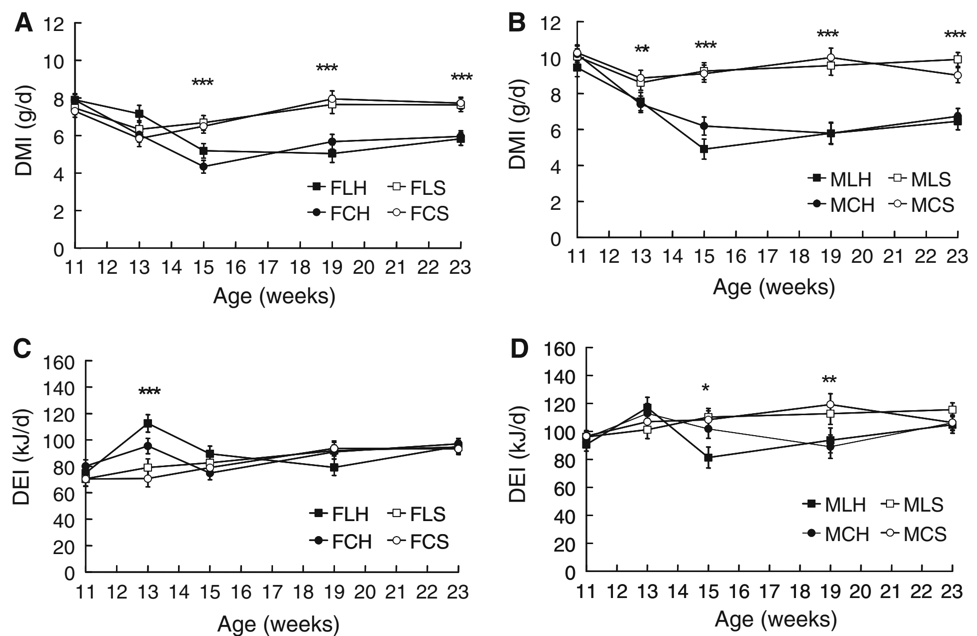

Fig. 3 Effects of maternal leptin treatment and high-fat diet on dry matter and digestible energy intake of the offspring. Dry matter intake (DMI) in female (**a**) and male (**b**) offspring aged 11, 13, 15, 19 and 23 weeks, and digestible energy intake (DEI) in female (**c**) and male (**d**) offspring aged 11, 13, 15, 19 and 23 weeks. Values are least square mean ± SE ($n = 9$–13) from two-way ANCOVA with body mass as covariate. $^{*}P < 0.05$ *H* versus *S* offspring; $^{**}P < 0.01$ *H* versus *S* offspring; $^{***}P < 0.001$ *H* versus *S* offspring. *F* female, *M* male, *L* maternal leptin treatment, *C* maternal control treatment, *H* offspring high-fat diet, *S* Offspring standard (low-fat) diet

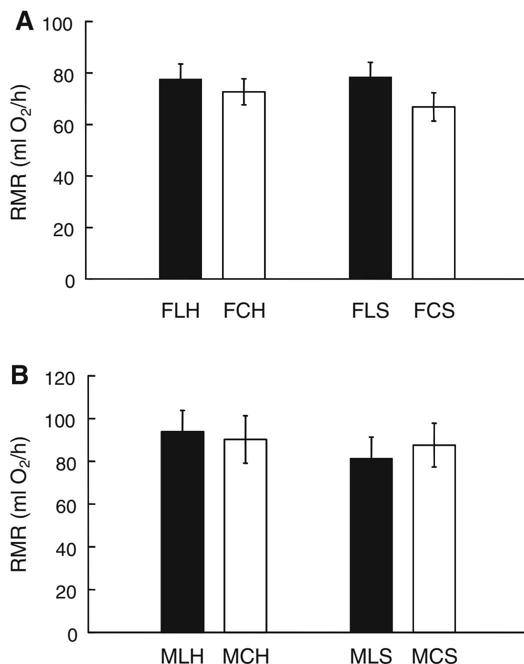

Fig. 4 Effects of maternal leptin treatment and high-fat diet on energy metabolism of the offspring. Resting metabolic rate (RMR) in female (**a**) and male (**b**) offspring at 22 weeks of age. Values are least square mean ± SE ($n = 6$–8) from two-way ANCOVA with body mass as covariate

neonatal leptin administration resulting in higher body mass and increased serum leptin concentration in adult rats (Cravo et al. 2002), which led to leptin resistance by reducing the expression of the hypothalamic leptin receptor (Toste et al. 2006). Similar effects were also detected in mice (Yura et al. 2005). These contradictory results in different studies of neonatal leptin treatment may mean that there are duration and dosage effects involved. There is some evidence that there are critical time windows when effects of developmental programming are most important (Stocker and Cawthorne 2008). Men who were exposed to the Dutch Hunger Winter famine in 1944 during fetal life had significantly disparate obesity rates if they were exposed to the famine during different prenatal periods (Ravelli et al. 1976). Further studies are required to substantiate that the timing and duration of leptin exposure are critical for early life programming in different species.

Cui et al. (2011) study showed that the dose of leptin used in the present study reduced food intake and body mass in lactating voles, but did not affect daily energy expenditure, milk production or pup growth. Since leptin administration changed hypothalamic gene expression during cold adaptation (Tang et al. 2009) and lactation (Cui et al. 2011) in voles, in-depth studies are needed to identify the effect of leptin administration on developmental programming of the hypothalamus in Brandt's voles.

Wade (1982) stated that, in golden hamsters, high-fat feeding can lead to obesity in the absence of hyperphagia. A limited number of studies in rats gave similar results (Oscai et al. 1987). Male voles fed a high-fat diet showed a reduction in digestible energy intake after 4–8 weeks. Zhao et al (2010) also found that the adult Brandt's vole under short-day acclimation was not resistant to high-fat diet-induced obesity. It seems that hyperphagia is not necessary for high-fat diet-induced body mass gain or obesity. Our data further indicate that, even with lower digestible energy intake, a high-fat diet can also induce increase in body mass. Unlike most other animal models, voles fed a high-

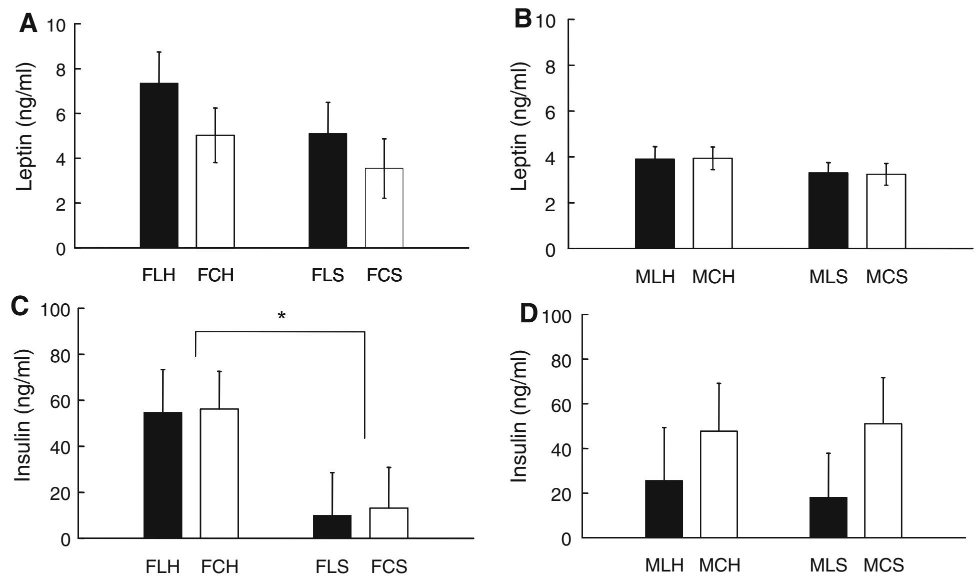

Fig. 5 Effects of maternal leptin treatment and high-fat diet on terminal serum hormone concentrations of the offspring. Serum leptin (**a**, **b**) and insulin (**c**, **d**) in female (**a**, **c**) and male (**b**, **d**) offspring. Values are mean ± SE ($n = 9–13$). $*P < 0.05$ *F* versus *S* offspring; $***P < 0.001$ *F* versus *S* offspring (two-way ANOVA). *F* female, *M* male, *L* maternal leptin treatment, *C* maternal control treatment, *H* high-fat diet, *S* standard (low-fat) diet

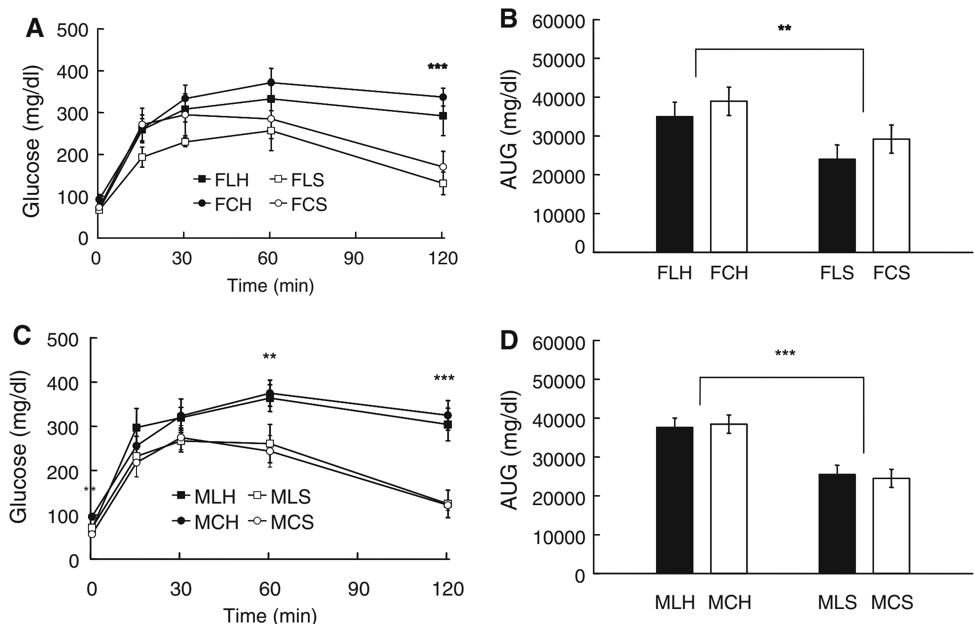

Fig. 6 Effects of maternal leptin treatment and high-fat diet on glucose tolerance of the offspring. Blood glucose concentrations (**a**, **c**) and area under the curve (AUC) (**b**, **d**) during the IGTT in female (**a**, **b**) and male (**c**, **d**) offsping at 21 weeks of age. Values are mean ± SE ($n = 8$). $**P < 0.01$ *H* versus *S* offspring; $***P < 0.001$ *H* versus *S* offspring (two-way ANOVA). *F* female, *M* male, *L* maternal leptin treatment, *C* maternal control treatment, *H* high-fat diet, *S* standard (low-fat) diet

fat diet exhibited reduced digestible energy intake and no change in resting metabolic rate, suggesting that they probably reduced their activity level in response to the high-fat diet.

The response to high-fat diet-induced-obesity seems to be sex dependent, since obesity is generally more prevalent in women than men (Lovejoy and Sainsbury 2009). In experimental model animals, some studies have shown that cafeteria feeding induces a higher body mass gain in female than in male rats (Rodriguez et al. 2001). Our results also show sex differences in response to the high-fat diet in Brandt's voles, with females having greater capacity for body fat accumulation and higher serum insulin concentrations. This may be attributed, in part, to greater digestible energy intake in female voles as compared with males.

A high-fat diet is known to increase body mass and fat mass and to lead to lower glucose tolerance and insulin resistance in rats, mice, and even humans (Amiel et al. 1986; Brown et al. 2002; Winzell and Ahren 2004). Both female and male voles fed on the high-fat diet displayed lower glucose tolerance, despite the increased insulin concentration in the females, indicative of the emergence of insulin resistance. Recent studies have demonstrated that visceral fat has more influence on insulin resistance than subcutaneous fat (Cnop et al. 2002; Kelley et al. 2000;

Westphal 2008). In agreement with these results, the high-fat diet resulted in lower glucose tolerance with increased epididymal fat in male offspring in our study.

In summary, the present study shows that, in Brandt's voles, maternal leptin treatment during peak lactation did not affect the body mass, energy homeostasis, diet-induced-obesity, or glucose tolerance in their offspring. Female voles accumulated more body fat and had higher insulin concentrations than males when fed a high-fat diet. With reduced energy intake, male voles fed the high-fat diet had greater body mass and glucose intolerance. Further study into the physiological and molecular mechanisms in response to high-fat diets should increase our understanding of diet-induced obesity and insulin resistance in Brandt's voles.

Acknowledgments Thanks to Professor John R. Speakman, University of Aberdeen, Scotland, UK and Professor Ian D. Hume, University of Sydney, Australia, for their constructive suggestions and improvement of the English expression. We are grateful to all the members of Animal Physiological Ecology Group for their help on the experiment and discussion for the results. This study was supported by the National Natural Scientific Foundation of China (No.30625009), National Basic Research Program of China (2007BC109103), and the Chinese Academy of Sciences (KSCX2-YW-N-06 and KSCX2-EW-N-05) to DHW.

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