

# Effects of dietary fiber content on energetics in nonreproductive and reproductive Brandt's voles (*Lasiopodomys brandtii*)

Mei-Fang Lou, Xue-Ying Zhang, Rong-Shu Fu, and De-Hua Wang

**Abstract:** Food quality can affect many physiological characteristics in small mammals. Reproduction is a highly energy-demanding period especially for the females to produce and feed their offspring. We hypothesized that energy intake was constrained at different levels in nonreproductive and reproductive females and thus they adopted diverse energy strategies in response to diet changes. Here, we tested the effects of low fiber diet (3.5% vs. 12.4%) on energy intake and thermogenesis in nonreproductive and reproductive Brandt's voles (*Lasiopodomys brandtii* (Radde, 1861)), a herbivorous species. We found that the voles decreased food intake while keeping a stable digestible energy intake (DEI) in response to the low fiber diet, but DEI was increased in reproductive voles at peak lactation. Uncoupling protein 1 content in brown adipose tissue decreased in nonreproductive voles, but was stable in reproductive voles on the low fiber diet. Litter mass on day 18 of age tended to increase in the low fiber group compared with that in the control group. Our findings demonstrate that the voles have a target intake to maintain energy balance when diet composition changes and energy intake may be constrained at a high level for the reproductive voles to improve their offspring's fitness.

**Key words:** Brandt's voles, *Lasiopodomys brandtii*, energy intake, leptin, low fiber diet, reproductive output.

**Résumé :** La qualité de la nourriture peut avoir une incidence sur de nombreuses caractéristiques physiologiques des petits mammifères. La reproduction est une période de forte demande énergétique, particulièrement pour les femelles, qui doivent produire et nourrir leur progéniture. Nous postulons que l'apport énergétique nécessaire s'établit à différents niveaux chez les femelles non reproductrices et reproductrices, et que les femelles adoptent donc diverses stratégies énergétiques en réponse aux variations de leur régime alimentaire. Nous vérifions les effets d'un régime pauvre en fibres (3,5 % contre 12,4 %) sur l'apport énergétique et la thermogénèse chez des campagnols de Brandt (*Lasiopodomys brandtii* (Radde, 1861)), une espèce herbivore. Nous avons constaté que les campagnols réduisaient leur apport alimentaire tout en maintenant un apport d'énergie digestible (DEI) stable en réaction au régime pauvre en fibres, mais que le DEI augmentait chez les campagnols reproducteurs durant le maximum de lactation. La teneur en protéine découplante 1 dans les tissus adipeux bruns diminuait chez les campagnols non reproducteurs, mais était stable chez les campagnols reproducteurs soumis au régime pauvre en fibres. La masse de la portée au 18<sup>e</sup> jour tendait à augmenter dans le groupe traité au régime pauvre en fibres par rapport à celle du groupe témoin. Nos résultats démontrent que les campagnols ont un apport cible permettant de maintenir leur équilibre énergétique quand la composition du régime alimentaire change et que l'apport énergétique pourrait devoir être maintenu à un niveau élevé pour permettre aux campagnols reproducteurs d'améliorer l'aptitude de leur progéniture. [Traduit par la Rédaction]

**Mots-clés :** campagnol de Brandt, *Lasiopodomys brandtii*, apport énergétique, leptine, régime pauvre en fibres, efficacité de la reproduction.

## Introduction

Food quality can affect many physiological characteristics in small mammals, such as body mass, body fat content, energy metabolism, thermogenesis, and reproductive traits (McNab 1986; Bozinovic 1995). Fiber is one of the components of food that is difficult to digest, thus affecting overall digestibility and the rate of energy metabolism (Van Soest et al. 1991). Because of the high metabolic rate, small mammals, which feed mainly on green parts of plants, should not select high fiber food but select food of high quality to eat (Sinclair et al. 1982; Foley and Cork 1992). However, high-quality food is not often available in the field and therefore many small mammals such as herbivores have evolved to feed on

the high fiber food. These species usually compensate for the low-quality food by increased food intake and a combination of changes in gut capacity and rapid turnover time of food (Hammond and Wunder 1991; Bozinovic et al. 1997; Owl and Batzli 1998; McNab 2002; Zhao and Wang 2007). In addition, some species such as the degu (*Octodon degus* (Molina, 1782)) decreased basal metabolic rate (BMR) or resting metabolic rate (RMR) to reduce energy expenditure (Velo and Bozinovic 1993), but others such as Darwin's leaf-eared mouse (*Phyllotis darwini* (Waterhouse, 1837)) and Brandt's vole (*Lasiopodomys brandtii* (Radde, 1861)) did not change BMR in response to the high fiber diet (Bozinovic and Novoa 1997; Zhao and Wang 2007).

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Reproduction is a period with increased nutritional and energetic requirements for mammals (Bronson 1985; Clutton-Brock et al. 1989; Speakman 2008). To meet these requirements, small mammals have evolved several major metabolic adaptations, such as increasing food intake and gut mass, decreasing thermogenesis, and using fat deposits during lactation (Speakman 2008; Zhang and Wang 2008; Derrickson 2013). The maximum energy intake was physiologically constrained, although the mothers tried to increase energy intake at peak lactation (Speakman 2008). The decrease in thermogenesis during lactation is indicated by decreased expression of uncoupling protein 1 (UCP1), which is uniquely expressed in the inner membrane of brown adipose tissue (BAT) and functions to uncouple oxidative metabolism from ATP production (Cannon and Nedergaard 2004). Leptin is mainly secreted by adipocytes and plays a critical role via a negative feedback pathway in regulating energy intake and body mass (Friedman and Halaas 1998). Serum leptin increased during pregnancy and decreased during lactation (Zhang and Wang 2008). The decrease in serum leptin during lactation would stimulate food intake to meet the high energy demands by acting on the receptors in the hypothalamus. Prolactin is greatly secreted during pregnancy and lactation and functions to stimulate milk production and regulate food intake (Grattan 2002).

Brandt's voles are typical steppe herbivores that primarily inhabit the Inner Mongolian grasslands of China, the Republic of Mongolia, and the region of Baikal in Russia (Zhang and Wang 1998). The food habit of Brandt's voles and fiber content of food showed seasonal changes in the field (Wang et al. 1992). The previous studies showed that male and female Brandt's voles increased food intake to keep a stable digestible energy intake (DEI), but decreased body mass in response to the high fiber diet under nonbreeding condition (Pei et al. 2001; Song and Wang 2006; Zhao and Wang 2007). Breeding females have high energetic demands for producing and feeding their offspring, and commonly maximize their reproductive fitness (Clutton-Brock et al. 1989). We hypothesized that energy intake was constrained at different levels in nonreproductive and reproductive voles and thus they adopted diverse energy strategies in response to diet changes. We measured the adaptive changes in physiological (body mass, food intake, RMR, and body composition), hormonal (leptin and prolactin), and molecular (UCP1 expression in BAT) markers to distinguish the different metabolic strategies at several levels between nonreproductive and reproductive Brandt's voles in response to low fiber diet. Based on the above hypothesis, we predicted that energy intake would be constrained at a high level during peak lactation and that the voles on the low fiber diet ate as much but benefited from the higher DEI/g of food that they ate.

## Materials and methods

### Animals

Brandt's voles were the offspring of our laboratory colony, which was originally trapped from Inner Mongolian grasslands in 1999. Room temperature was constant at  $23 \pm 1^\circ\text{C}$  with a 16 h light : 8 h dark photoperiod (lights on at 0400). The voles were weaned at 21 days and then housed in same-gender sibling pairs in plastic cages (30 cm  $\times$  15 cm  $\times$  20 cm) with sawdust as beddings.

### Experimental design

Brandt's voles are herbivores and thus were fed a relative higher fiber diet (rabbit pellet chow, 12.4% fiber) compared with other rodents such as mice and rats (rat pellet chow, 3.5% fiber) (Zhao and Wang 2007; Maurer and Reimer 2011). The present study was designed to test how the voles responded to a relatively lower fiber diet (3.5% fiber). At 3–4 months of age, 34 virgin females (weighing 30–40 g) were moved into individual cages for at least 2 weeks before the experiment. According to a two-factor (dietary fiber and reproduction) design, the animals were randomly as-

**Table 1.** Nutrient composition of control diet and the low fiber diet fed to Brandt's voles (*Lasiopodomys brandtii*).

Nutrient	Control diet	Low fiber diet
Gross energy (kJ·g <sup>-1</sup> )	17.5	17.4
Crude protein (made of casein; %)	17.7	17.0
Crude fat (made of soybean oil; %)	2.7	3.0
Crude fiber (made of cellulose; %)	12.4	3.5
Carbohydrate (made of starch; %)	47.0	55.9
Ash (%)	8.7	8.7

signed to four experimental regimens: nonreproduction with standard rabbit pellet chow (control diet, 12.4% fiber, NCon,  $n = 8$ ), nonreproduction with a low fiber diet (3.5% fiber, NLF,  $n = 8$ ), reproduction with standard rabbit pellet chow (RCon,  $n = 9$ ), and reproduction with a low fiber diet (RLF,  $n = 9$ ). Only the mothers with litter sizes of 5–9 were chosen in this study to remove the effect of extremely large or small litter sizes on maternal energetic strategies. Standard rabbit pellet chow and the low fiber diet were purchased from the same company (Beijing HFK Bioscience Co., Ltd., Beijing, People's Republic of China), and the detailed compositions of protein, fat, and fiber (Table 1) were analyzed with a Kjeltac 2100 (FOSS Analytical A/S, Hillerød, Denmark). Two weeks after acclimation to different diets, the voles were mated, and 21 days after mating, the pregnant voles gave birth. The day of parturition was designated as day 0 of lactation and lactation lasted for 21 days. The experiment was stopped on day 18 of lactation (peak lactation) after which it was hard to separate maternal food intake from offspring feeding. Body mass was measured once every 3 days. Food intake was measured every 6 days except during mating time. RMR and all molecular markers were measured at peak lactation when maternal energy demands were the highest of the entire reproductive period (Zhang and Wang 2008). The experiment was performed in accordance with the Animal Care and Use Committee of the Institute of Zoology, the Chinese Academy of Sciences.

### Energy intake

Energy intake was measured in metabolic cages. Food and water were provided in excess of the animals' needs. Food residues and feces were collected quantitatively from each subject over the last 3 days of every 6 days during diet acclimation, and separated after they were dried at  $60^\circ\text{C}$  to constant mass. Dry matter intake (DMI) was calculated from the difference between the dry mass of food that was provided and the food residue. Energy contents of the food and feces were determined with a Parr 1281 oxygen bomb calorimeter (Parr Instrument Company, Moline, Illinois, USA). Gross energy intake (GEI), DEI, and apparent digestibility of energy were calculated based on Grodzinski and Wunder (1975) and Liu et al. (2003):

$$\text{GEI (kJ/day)} = \text{DMI (g/day)} \times \text{energy content of food (kJ/g)}$$

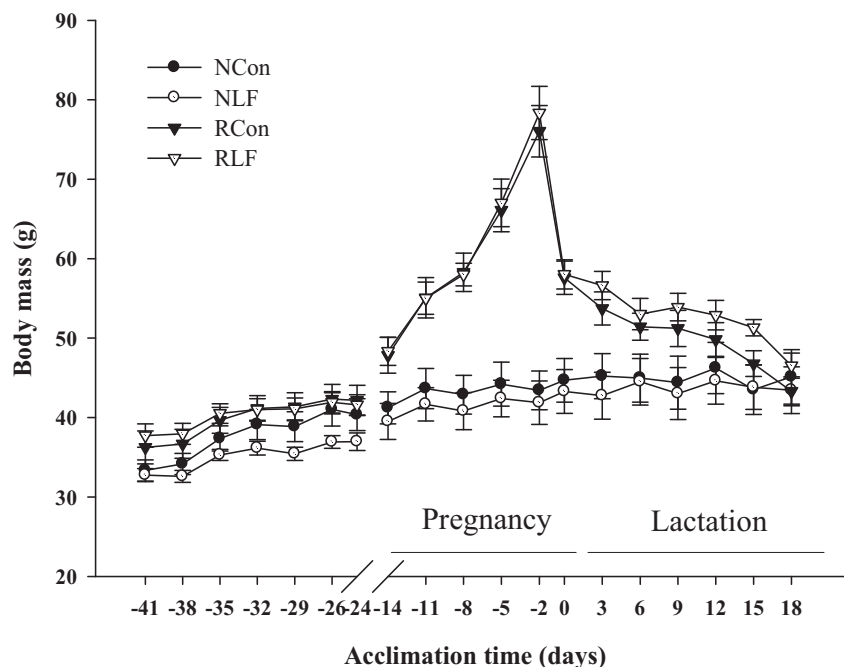
$$\text{DEI (kJ/day)} = \text{GEI} - \text{dry mass of feces (g/day)} \times \text{energy content of feces (kJ/g)}$$

$$\text{Digestibility (\%)} = (\text{DEI/GEI}) \times 100$$

### RMR measurement

Between the hours of 0700 and 2000, RMR was assessed at around  $30^\circ\text{C}$  (within their thermal neutral zone) (Wang et al. 2003) using the Sable TurboFOX Complete Field System (Li et al. 2010). Individual vole at the corresponding time on day 16 of lactation was placed in a metabolic chamber (200 mm  $\times$  130 mm  $\times$  85 mm) for 3 h. The flow rate of air (dried with anhydrous  $\text{CaSO}_4$ ; W.A. Hammond Drierite Co. Ltd., Xenia, Ohio, USA) was 600–800 mL/min. Gases leaving the chamber were subsampled and dried by ND-2 (Sable Systems, Las Vegas, Nevada, USA), then

**Fig. 1.** Effects of different dietary fiber content on body mass in nonreproductive and reproductive Brandt's voles (*Lasiopodomys brandtii*). NCon, nonreproduction with control diet ( $n = 8$ ); NLF, nonreproduction with low fiber diet ( $n = 8$ ); RCon, reproduction with control diet ( $n = 9$ ); RLF, reproduction with low fiber diet ( $n = 9$ ).



passed through the oxygen and carbon dioxide analyzers at approximately 100 mL/min. The rate of oxygen consumption was calculated as described previously (Li et al. 2010) and RMR was estimated from the stable lowest rate of oxygen consumption over 5 min.

$$VO_2 = \frac{FR \times (FiO_2 - FeO_2) - FR \times FeO_2 \times (FeCO_2 - FiCO_2)}{1 - FeO_2} \times 60$$

where FR is the flow rate (mL/min), Fi is the input O<sub>2</sub> or CO<sub>2</sub> concentration (%), and Fe is the excurrent O<sub>2</sub> or CO<sub>2</sub> concentration (%).

**Body composition analysis**

All subjects were weighed and sacrificed with CO<sub>2</sub> asphyxiation between the hours of 0900 and 1100 on day 18 of lactation or at the corresponding time for the nonreproductive group. Blood samples were collected and the interscapular BAT was carefully dissected, weighed, frozen in liquid nitrogen, and stored at -80 °C. The visceral organs, including heart, lung, liver, kidneys, spleen, gonad, and digestive tract (stomach, small intestine, caecum, and colon) were removed and weighed (±1 mg). The stomach and intestines were rinsed with saline to eliminate the contents. The mesenteric fat, epigonadal fat, retroperitoneal fat, and subcutaneous fat were also dissected carefully and weighed (±1 mg). 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 wet and dry carcass masses was the water mass of the carcass (body water).

**Serum leptin and prolactin assays**

Serum leptin concentration was measured by radioimmunoassay (RIA) with the <sup>125</sup>I multispecies kit (catalogue No. XL-85K; Linco Research Inc., St. Charles, Missouri, USA) (Li and Wang 2005; Zhang and Wang 2006). The lowest and highest concentrations of leptin that can be detected by this assay were 1.0 and 50 ng/mL,

respectively, when using a 100 μL sample. The intra- and inter-assay variabilities for leptin RIA were <3.6% and 8.7%, respectively.

Serum prolactin concentration was determined using RIA kits from Beijing Institute of Northern Biotech. The intra- and inter-assay variabilities were 4.3% and 7.6%, respectively.

**UCP1 measurement**

UCP1 content was measured by Western blotting as described previously (Li and Wang 2005; Zhang and Wang 2006). Total BAT protein (80 μg per lane) determined by the Folin phenol method was separated in a discontinuous sodium dodecyl sulfate – polyacrylamide gel (10% running gel and 4% stacking gel) and transferred onto a PVDF membrane (IPVH00010; EMD Millipore, Billerica, Massachusetts, USA) (200 mA, 2 h); the membrane was blocked in 5% nonfat dry milk in Tris-buffered saline – Tween for 1 h at room temperature. UCP1 was detected using a polyclonal rabbit anti-hamster UCP1 (1:5000) as the primary antibody, peroxidase-conjugated sheep anti-rabbit IgG (1:5000) as the secondary antibody, and then enhanced by chemoluminescence (Amersham ECL; GE Healthcare Life Sciences). UCP1 content was expressed as a relative unit (RU), as determined from area readings using the Quantity One software version 4.4.0 (Bio-Rad Laboratories, Inc., Hercules, California, USA).

**Data analysis**

Data were analyzed using SPSS version 17.0 software package (SPSS Inc., Chicago, Illinois, USA). Data on litter mass, litter size, and sex ratio were analyzed by independent-samples *t* test. Differences in body mass and digestibility were analyzed using repeated measurements and two-way ANOVA (dietary fiber and reproduction). Differences in DMI and DEI among groups were analyzed by repeated measurements and two-way ANCOVA with body mass as the covariate. RMR and the mass of organs and white fat were analyzed by two-way ANCOVA with body mass as the covariate. Body composition, UCP1 content in BAT, and serum leptin and prolactin concentrations were analyzed by two-way ANOVA. Pearson correlation analysis was used to detect possible associations of



serum leptin and prolactin concentrations with body mass and DEI. Results are presented as means  $\pm$  SE and  $P < 0.05$  was considered to be statistically significant.

## Results

### Body mass

Prior to dietary acclimation, there was no significant difference in body mass among the four groups (day -41 and day -38,  $P > 0.05$ ; Fig. 1). Body mass showed a significant change with time ( $F_{[18,540]} = 146.806$ ,  $P < 0.001$ ), and was affected by reproduction ( $F_{[1,30]} = 23.077$ ,  $P < 0.001$ ) and by the interaction between reproduction and time ( $F_{[18,540]} = 57.452$ ,  $P < 0.001$ ), but was not influenced by dietary fiber ( $F_{[1,30]} = 0.027$ ,  $P > 0.05$ ) or the interaction between reproduction and dietary fiber ( $F_{[1,30]} = 0.693$ ,  $P > 0.05$ ). Reproductive voles showed higher body mass than nonreproductive voles from day -14 to day 15 ( $P < 0.05$ ; Fig. 1).

### Energy intake

Prior to diet acclimation, DMI, DEI, and digestibility were similar among the four groups (day -38,  $P > 0.05$ ; Figs. 2A–2C). DMI was significantly affected by dietary fiber ( $F_{[1,29]} = 12.335$ ,  $P < 0.01$ ), reproduction ( $F_{[1,29]} = 8.650$ ,  $P < 0.01$ ), the interaction between reproduction and time ( $F_{[7,203]} = 20.735$ ,  $P < 0.001$ ), and the interaction between diet and time ( $F_{[7,203]} = 3.848$ ,  $P < 0.01$ ), but was not affected by the interaction between reproduction and dietary fiber ( $F_{[1,29]} = 0.351$ ,  $P > 0.05$ ). For the nonreproductive females, DMI of low fiber diet was lower than that of the control group; for the reproductive females, DMI of low fiber diet was lower than that of the control group before delivery ( $P < 0.01$ ) and there was no difference after delivery. DMI of reproductive voles was significantly higher than that of nonreproductive voles from day 12 to day 18 of lactation ( $P < 0.01$ ; Fig. 2A). DEI was significantly higher in reproductive voles than in nonreproductive voles ( $F_{[1,29]} = 9.826$ ,  $P < 0.01$ ), but was not affected by dietary fiber during the entire reproductive process ( $F_{[1,29]} = 0.294$ ,  $P > 0.05$ ). Interestingly, the low fiber diet resulted in a significant increase (by 28%, equivalent to 45 kJ in energy content) in DEI in reproductive voles only on day 18 of lactation ( $P < 0.05$ ), but did not in other reproductive stages or in the nonreproductive group ( $P > 0.05$ ; Fig. 2B). Digestibility increased in the first week after the diet change and then kept stable ( $F_{[7,210]} = 34.602$ ,  $P < 0.001$ ). Digestibility increased in both nonreproductive and reproductive voles fed the low fiber diet ( $F_{[1,30]} = 418.012$ ,  $P < 0.001$ ; Fig. 2C), but was not affected by the interaction between reproduction and dietary fiber ( $F_{[1,30]} = 1.250$ ,  $P > 0.05$ ).

### Body composition and organ mass

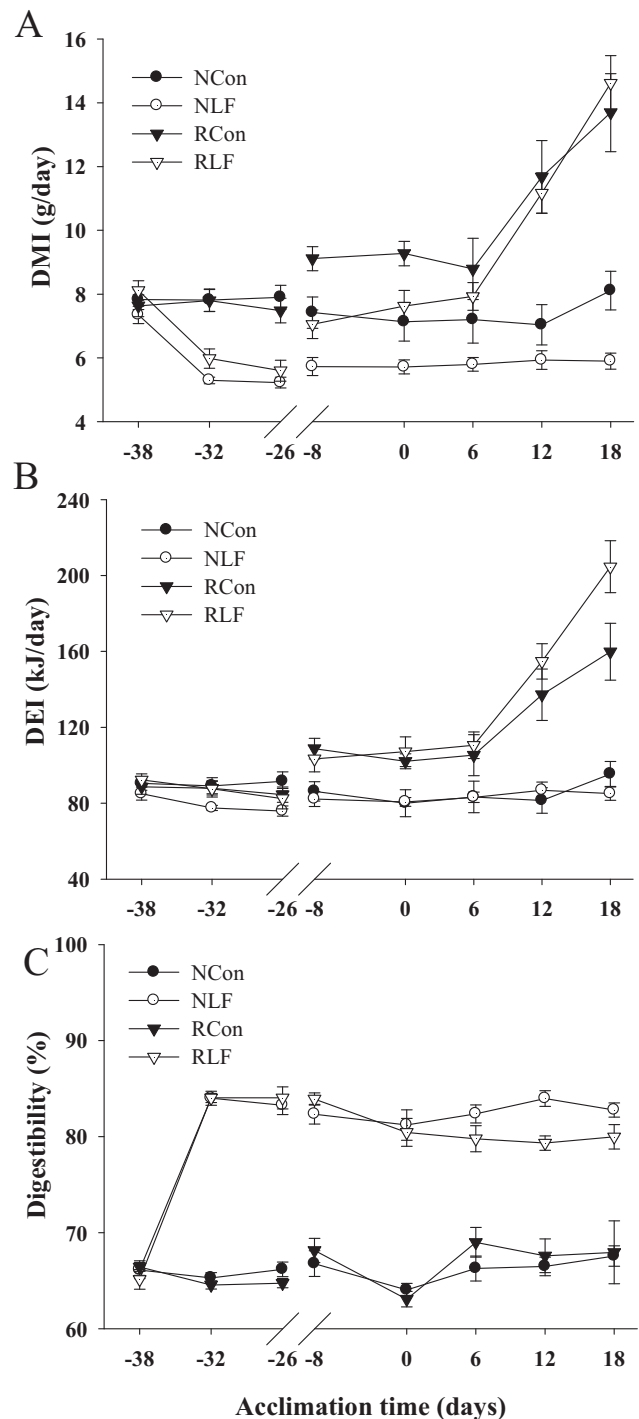
The reproductive voles had heavier liver, kidneys, and digestive tract (stomach, small intestine, caecum, and colon), but lighter BAT, white fat pads (mesenteric fat, epigonadal fat, retroperitoneal fat, and subcutaneous fat), and carcass than nonreproductive voles ( $P < 0.05$ ; Tables 2, 3). Low fiber diet resulted in higher masses of spleen (dry), liver (wet), and small intestine with content, but lower mass of caecum compared with the control diet ( $P < 0.05$ ; Tables 2, 3). Among all the composition and organs, only the dry mass of stomach was affected by the interaction between reproduction and dietary fiber ( $F_{[1,29]} = 4.205$ ,  $P < 0.05$ ; Table 3).

### RMR and UCPI content in BAT

The reproductive voles had higher RMR than nonreproductive voles ( $F_{[1,29]} = 11.534$ ,  $P < 0.01$ ). RMR was not affected by dietary fiber ( $F_{[1,29]} = 0.688$ ,  $P > 0.05$ ) or the interaction between dietary fiber and reproduction ( $F_{[1,29]} = 0.550$ ,  $P > 0.05$ ; Fig. 3A).

Reproductive voles decreased UCPI content in BAT compared with nonreproductive voles ( $F_{[1,30]} = 21.334$ ,  $P < 0.001$ ). UCPI content in BAT was affected by the interaction between reproduction and dietary fiber, with a decrease in nonreproductive voles but

**Fig. 2.** Effects of different dietary fiber content on dry matter intake (DMI; A), digestible energy intake (DEI; B), and digestibility (C) in nonreproductive and reproductive Brandt's voles (*Lasiopodomys brandtii*). NCon, nonreproduction with control diet ( $n = 8$ ); NLF, nonreproduction with low fiber diet ( $n = 8$ ); RCon, reproduction with control diet ( $n = 9$ ); RLF, reproduction with low fiber diet ( $n = 9$ ).



with no change in reproductive voles fed the low fiber diet ( $F_{[1,30]} = 4.642$ ,  $P < 0.05$ ; Fig. 3B).

### Serum leptin and prolactin concentrations

Serum leptin was lower ( $F_{[1,30]} = 4.992$ ,  $P < 0.05$ ; Fig. 4A), but serum prolactin was higher ( $F_{[1,30]} = 19.663$ ,  $P < 0.001$ ; Fig. 4B), in reproductive voles than in nonreproductive voles. Neither serum

**Table 2.** Effects of different dietary fiber content on body composition and organ mass in nonreproductive and reproductive Brandt's voles (*Lasiopodomys brandtii*).

Parameter	Nonreproduction		Reproduction		Statistical summary
	Control diet (n = 8)	Low fiber diet (n = 8)	Control diet (n = 9)	Low fiber diet (n = 9)	
<b>Body composition</b>					
Final body mass (g)	45.1±3.4	43.4±2.9	43.3±1.8	46.5±1.6	
Wet carcass (g)	29.3±2.2a	27.8±2.0ab	24.3±1.0b	25.2±0.9ab	R*
Dry carcass (g)	14.4±1.5a	13.7±1.7a	9.1±0.6b	9.0±0.3b	R***
Body water (g)	14.8±0.9	14.1±0.6	15.2±0.4	16.2±0.7	
Mesenteric fat (g)	0.354±0.066a	0.310±0.043ab	0.233±0.016b	0.275±0.017b	R**
Epigonadal fat (g)	0.520±0.101a	0.529±0.138a	0.159±0.039b	0.131±0.025b	R***
Retroperitoneal fat (g)	0.690±0.152a	0.744±0.260a	0.116±0.036b	0.074±0.025b	R***
Subcutaneous fat (g)	2.558±0.518a	2.472±0.609a	0.390±0.134b	0.304±0.061b	R***
Total WAT (g)	4.123±0.779a	4.056±1.019a	0.899±0.213b	0.770±0.308b	R***
<b>Organ mass</b>					
BAT mass (g)	0.199±0.026a	0.202±0.029a	0.088±0.009b	0.115±0.008b	R***
Heart (g)					
Wet mass	0.189±0.011	0.190±0.009	0.191±0.011	0.207±0.009	
Dry mass	0.046±0.003	0.048±0.002	0.046±0.003	0.052±0.002	
Liver (g)					
Wet mass	1.502±0.116a	1.850±0.174ab	2.114±0.138b	2.537±0.132c	R***; D*
Dry mass	0.440±0.035a	0.537±0.052ab	0.598±0.044b	0.649±0.077b	R*
Spleen (g)					
Wet mass	0.052±0.025	0.032±0.003	0.031±0.002	0.037±0.003	
Dry mass	0.006±0.001a	0.009±0.001b	0.007±0.001ab	0.009±0.001b	D**
Lung (g)					
Wet mass	0.263±0.025	0.254±0.014	0.221±0.015	0.295±0.035	
Dry mass	0.064±0.007	0.065±0.005	0.051±0.004	0.070±0.007	
Kidneys (g)					
Wet mass	0.413±0.022ab	0.397±0.013a	0.441±0.017bc	0.468±0.012c	R**
Dry mass	0.105±0.006	0.101±0.003	0.105±0.005	0.109±0.004	
Gonad (g)					
Wet mass	0.128±0.024	0.106±0.014	0.103±0.013	0.117±0.013	
Dry mass	0.033±0.006	0.031±0.005	0.027±0.002	0.030±0.003	

**Note:** Gonad mass was the total mass of uterus and ovary. Values are expressed as means ± SE. Different letters in each row indicate significant difference ( $P < 0.05$ ). R, reproduction; D, dietary fiber; WAT, white adipose tissue; BAT, brown adipose tissue. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

**Table 3.** Effects of different dietary fiber content on the mass of digestive tract in nonreproductive and reproductive Brandt's voles (*Lasiopodomys brandtii*).

Parameter	Nonreproduction		Reproduction		Statistical summary
	Control diet (n = 8)	Low fiber diet (n = 8)	Control diet (n = 9)	Low fiber diet (n = 9)	
<b>Stomach</b>					
Mass with content (g)	0.870±0.166ab	0.693±0.083a	1.023±0.108bc	1.224±0.080c	R**
Wet mass (g)	0.258±0.023ab	0.226±0.012a	0.292±0.012bc	0.325±0.011c	R***
Dry mass (g)	0.061±0.004ab	0.055±0.003a	0.067±0.002bc	0.075±0.003c	R***; R × D*
<b>Small intestine</b>					
Mass with content (g)	1.399±0.128a	1.511±0.094a	2.029±0.151b	2.481±0.152c	R***; D*
Wet mass (g)	0.390±0.033a	0.427±0.044a	0.507±0.046ab	0.666±0.094b	R**
Dry mass (g)	0.076±0.008	0.088±0.011	0.084±0.009	0.109±0.016	
<b>Caecum</b>					
Mass with content (g)	2.250±0.214a	2.224±0.271a	4.842±1.269b	5.059±0.432b	R**
Wet mass (g)	0.417±0.036ab	0.299±0.034a	0.589±0.081c	0.500±0.020bc	R**; D*
Dry mass (g)	0.063±0.005a	0.054±0.006a	0.080±0.008b	0.066±0.003ab	R*; D*
<b>Colon</b>					
Mass with content (g)	0.626±0.053a	0.610±0.080a	1.158±0.098b	1.390±0.082c	R***
Wet mass (g)	0.293±0.031a	0.288±0.031a	0.399±0.044b	0.411±0.040b	R**
Dry mass (g)	0.060±0.006ab	0.056±0.006a	0.073±0.006b	0.073±0.005ab	R*

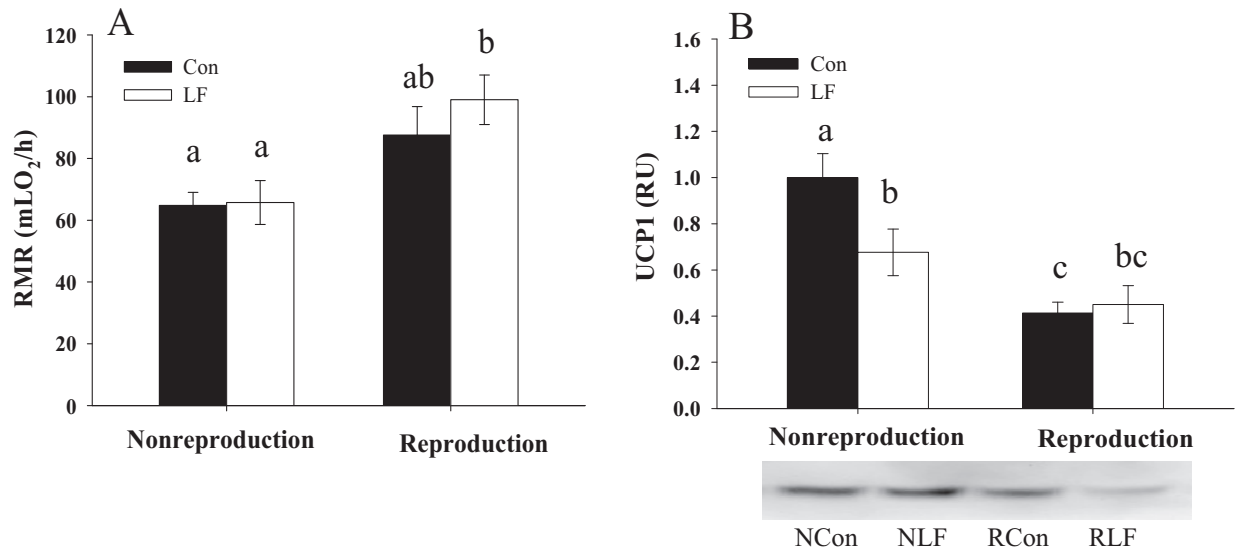
**Note:** Values are expressed as means ± SE. Different letters in each row indicate significant difference ( $P < 0.05$ ). R, reproduction; D, dietary fiber. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

leptin nor prolactin was affected by low fiber diet ( $P > 0.05$ ) or the interaction between reproduction and dietary fiber ( $P > 0.05$ ). Furthermore, serum leptin was negatively correlated ( $r = -0.362$ ,  $P < 0.05$ ) and serum prolactin was positively correlated ( $r = 0.372$ ,  $P < 0.05$ ) with DEI at peak lactation.

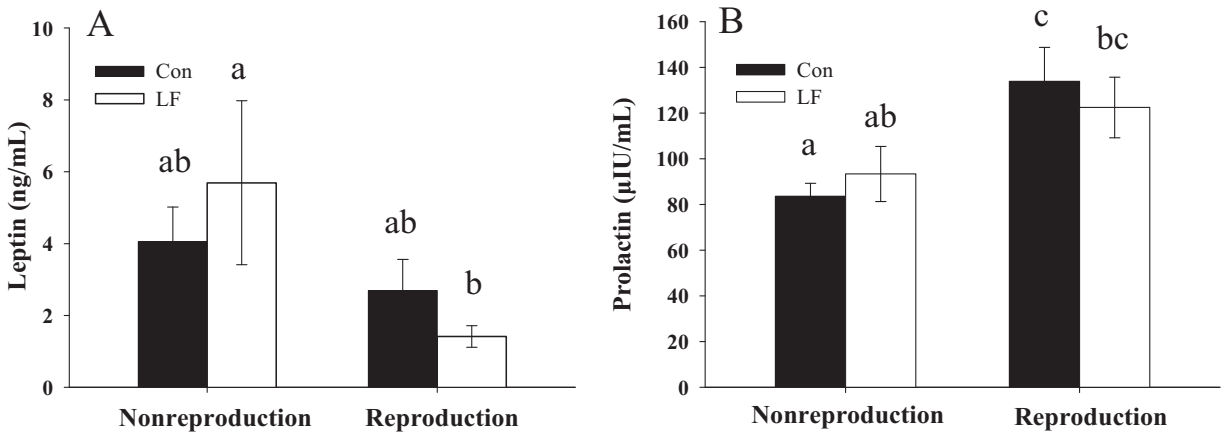
**Reproductive output**

Maternal low fiber diet resulted in a tendency to increase in litter mass on day 18 of lactation (by 24.5%,  $P = 0.095$ ), but did not affect litter size, sex ratio (the ratio of male to female), or survival rate of offspring during lactation ( $P > 0.05$ ; Table 4).

**Fig. 3.** Effects of different dietary fiber content on resting metabolic rate (RMR; A) and uncoupling protein 1 (UCP1) content in brown adipose tissue (BAT; B) in nonreproductive and reproductive Brandt's voles (*Lasiopodomys brandtii*). The gel strip in panel B represented the Western blotting detection of UCP1 content (32 kD) of the four groups. NCon, nonreproduction with control diet ( $n = 8$ ); NLF, nonreproduction with low fiber diet ( $n = 8$ ); RCon, reproduction with control diet ( $n = 9$ ); RLF, reproduction with low fiber diet ( $n = 9$ ); RU, relative unit. The significant differences ( $P < 0.05$ ) are indicated by the different lowercased letters.



**Fig. 4.** Effects of different dietary fiber content on serum leptin (A) and prolactin (B) concentrations in nonreproductive and reproductive Brandt's voles (*Lasiopodomys brandtii*). Con, control diet; LF, low fiber diet; IU, international unit. The sample size is 8–9 per group. The significant differences ( $P < 0.05$ ) are indicated by the different lowercased letters.



## Discussion

The physiological responses to the low fiber diet were examined in nonreproductive and reproductive voles in the present study. We found that in response to the low fiber diet, the nonreproductive voles decreased food intake but kept a stable DEI, while the reproductive voles kept a stable food intake but increased DEI at peak lactation. Litter mass at peak lactation tended to increase in the low fiber group compared with the control group. The data suggested that the reproductive voles at peak lactation obtained more energy while keeping a stable food intake from the easily digestible food to improve offspring fitness, whereas the nonreproductive voles kept a stable energy intake.

Small mammals living in the temperate zone are faced with seasonal environments, especially variations in food quality, and thus show seasonal body mass in the wild (McNab 1986; Bozinovic et al. 1997; Li and Wang 2005). For the herbivorous rodents, the change in dietary fiber content is an important factor inducing seasonal variations in body mass, food intake, and thermogenesis (Bozinovic 1995; Bozinovic et al. 1997). The laboratory studies

**Table 4.** Effects of different maternal dietary fiber content consumed by Brandt's voles (*Lasiopodomys brandtii*) on reproductive output.

Parameter	Control diet ( $n = 9$ )	Low fiber diet ( $n = 9$ )	Statistical summary
<b>Litter mass (g)</b>			
Day 0	19.3±1.38	22.2±1.00	ns
Day 18	80.0±8.47	99.6±4.41	0.095
<b>Litter size</b>			
Day 0	7±0.6	8±0.5	ns
Day 18	7±0.6	8±0.4	ns
<b>Sex ratio</b>	1.2±0.4	1.0±0.1	ns
<b>Survival rate (%)</b>	94.0	95.9	ns

Note: Values are expressed as means ± SE. ns, not significant.

showed that many rodents, such as the herbivorous degu, fat sand rat (*Psammomys obesus* Cretzschmar, 1828), and Brandt's voles, increased food intake and maintained a stable DEI and body mass when they were fed the high fiber diet (Bozinovic et al. 1997;

Degen et al. 2000; Zhao and Wang 2007). Prairie voles (*Microtus ochrogaster* (Wagner, 1842)), however, still decreased body mass, although they increased food intake in response to the high fiber diet (Voltura and Wunder 1998). All these studies, which were performed in nonbreeding animals, suggested that rodents compensated for low digestibility of the high fiber diet by increasing food intake to keep a stable DEI. Likewise, we found that the voles decreased food intake and kept a stable DEI when fed the easily digestible food with low fiber content. The change in food intake was associated with plastic changes in the digestive tract, especially the increased caecum mass, with the high fiber diet (Gross et al. 1985; Bozinovic et al. 1997; Pei et al. 2001; Zhao and Wang 2007) and decreased with the low fiber diet in the present study. These results indicate that the nonbreeding voles would take a strategy to change food intake to obtain the same amount of DEI with changes in dietary fiber content.

Energy requirements increase with the process of pregnancy and lactation in small mammals (Speakman 2008). Most metabolic characters, such as body mass, food intake, and thermogenesis, changed during reproduction. The present data showed that food intake increased by 75% at peak lactation when the maximal energy intake occurred in reproductive voles compared with nonreproductive voles. The lactating Siberian hamsters (*Phodopus sungorus* (Pallas, 1773)) even increased food intake by three times at peak lactation (Bartness 1997). We also found that the digestive tract with content was 20.9% of body mass in the reproductive voles but was 11.4% of body mass in the nonreproductive voles, suggesting that the reproductive voles had higher digestive tract plasticity and thus had a high level of constraint to maximum energy intake than nonreproductive voles. In addition, we found that both nonreproductive and reproductive voles with the low fiber diet kept a stable DEI, which seems to suggest that the voles have a target intake to maintain energy balance when diet composition changes. However, the reproductive voles at peak lactation obtained more DEI to try to increase litter mass when they were fed the low fiber diet. Some previous studies showed that maternal diets with different protein, fiber, or energy content had a significant effect on litter mass or pup mass, but no effect on litter size or the mortality of pups (Speakman et al. 2001; Derrickson and Lowas 2007; Hallam and Reimer 2013). Other studies reported different results, namely, that maternal high fiber or protein diet imposed no effect on reproductive output but conferred a long-term effect on offspring fitness (Maurer and Reimer 2011; Lou et al. 2015). These findings suggest that maximum DEI was constrained at a high level and therefore the mothers at peak lactation ate as much and benefited from the higher DEI/g of food that they ate to improve their offspring's fitness when the diets with high quality were available.

UCP1 in BAT is an important indicator of BAT thermogenesis (Cannon and Nedergaard 2004). As the previous study reported (Zhang and Wang 2008), BAT mass and UCP1 content in BAT decreased during lactation, which may contribute to saving energy and avoiding hyperthermia (Speakman 2008). We found that UCP1 content decreased in nonreproductive voles but did not change in reproductive voles in response to the low fiber diet. BAT thermogenesis is the consequence of the transformation of the chemical energy from consumed food into heat (Rothwell and Stock 1979; Cannon and Nedergaard 2004). Therefore, the thermogenesis varied with the change in food intake when the voles were fed the low fiber diet. Neither nonreproductive nor reproductive voles changed RMR in response to the low fiber diet, which was consistent with the previous study showing no change in BMR in nonbreeding voles with the high fiber diet (Zhao and Wang, 2007). These data indicate that the voles would adjust food intake and thermogenesis to keep energy balance in response to diet changes.

Energy intake and thermogenesis are controlled by peripheral hormones. For example, leptin acts on the receptors in the hypo-

thalamus and regulates the expression of orexigenic and anorexigenic neuropeptides (Friedman and Halaas 1998). Another hormone, prolactin, is greatly secreted during pregnancy and lactation and is also involved in metabolic regulation during reproduction (Grattan 2002). We found that serum leptin and prolactin were negatively and positively related with DEI, respectively, but neither of them varied with diet change, which was supported by previous studies on voles (Zhao and Wang 2007; Lou et al. 2015). These data suggest that serum leptin and prolactin may be more related with the regulation of energy intake during reproduction than with diet change.

In conclusion, the reproductive Brandt's voles exhibited different adaptive energetics owing to a high level of constraint in energy intake compared with nonreproductive voles in response to the low fiber diet. Voles at peak lactation on the low fiber diet were able to increase DEI but did not increase food intake; the tendency towards increased pup growth suggests that the increase in DEI may result in improved offspring fitness. BAT thermogenesis varied with the change in food intake when the voles were fed the low fiber diet. These diverse energetic strategies enable small mammals to meet the energy demands that optimize their survival and reproduction. The exact mechanisms that result in different responses to food quality in nonreproductive and reproductive mammals deserve further investigations.

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