RESEARCH ARTICLE

Hypothalamic neuropeptides, not leptin sensitivity, contributes to the hyperphagia in lactating Brandt's voles, *Lasiopodomys brandtii*

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SUMMARY

Both pregnancy and lactation are associated with hyperphagia, and circulating leptin levels are elevated during pregnancy but decreased during lactation in Brandt's voles, *Lasiopodomys brandtii*. Previous findings suggest that impaired leptin sensitivity contributes to hyperphagia during pregnancy. The present study aimed to examine whether the decreased circulating leptin level and/or hypothalamic leptin sensitivity contributed to the hyperphagia during lactation in Brandt's voles. The serum leptin level and mRNA expression of the long form of the leptin receptor (Ob-Rb), suppressor-of-cytokine-signalling-3 (SOCS-3), neuropeptide Y (NPY), agouti-related protein (AgRP), pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) in the hypothalamus were examined on dioestrous, day 5, day 17 of lactation and day 27 (1 week after weaning) in Brandt's voles. Compared with controls, hypothalamic Ob-Rb and SOCS-3 mRNA expression was not significantly changed during lactation. The serum leptin level was significantly lower in lactating females than in the non-reproductive group. Hypothalamic NPY and AgRP mRNA expression significantly increased whereas POMC mRNA expression was significantly decreased during lactation compared with controls. However, there were no significant changes in hypothalamic CART mRNA expression. Food intake was positively correlated with NPY and AgRP mRNA expression but negatively correlated with POMC mRNA expression during lactation. These data suggest that hyperphagia during lactation was associated with low leptin levels, but not impaired leptin sensitivity, and that the hypothalamic neuropeptides NPY, AgRP and POMC are involved in mediating the role of leptin in food intake regulation in lactating Brandt's voles.

Key words: leptin, neuropeptide, Ob-Rb, suppressor-of-cytokine-signalling-3, SOCS-3, lactation, hyperphagia, Brandt's vole, *Lasiopodomys brandtii*.

INTRODUCTION

The energy requirements of mother rats are greatly increased during lactation because of milk production (Flint and Vernon, 1998). Lactating animals can match the high energetic cost by increasing food intake (Wade and Schneider, 1992; Zhang and Wang, 2007), reducing thermogenesis in brown adipose tissue (BAT) (Trayhurn and Richard, 1985; Smith and Grove, 2002; Zhang and Wang, 2007) and mobilizing body fat (Schneider and Wade, 1987; Barber et al., 1997). The characteristics of energy balance associated with lactation are extreme hyperphagia coupled with negative energy balance (Smith and Grove, 2002). However, the mechanisms of hyperphagia during lactation are not well known, especially for small wild mammals.

One possible cause of hyperphagia is the low level of leptin, which is secreted mainly by adipose tissue. The serum leptin concentration has been shown to decrease by 20–70% during lactation in rats (Kawai et al., 1997; Woodside et al., 2000; Denis et al., 2003a) and wild Brandt's voles (Zhang and Wang, 2007). Besides the low leptin concentration, decreased leptin sensitivity in the hypothalamus is another important cause of hyperphagia (El-Haschimi et al., 2000; Munzberg et al., 2004). Leptin acts *via* receptors in the hypothalamus to regulate appetite and energy expenditure (Friedman and Halaas, 1998). Many studies have indicated that a decrease in the mRNA expression of the long form of the leptin receptor (Ob-Rb) in the

hypothalamus impaired leptin sensitivity and induced hyperphagia in rats (e.g. Munzberg et al., 2005; Szczepankiewicz et al., 2006). Suppressor-of-cytokine-signalling-3 (SOCS-3) is a target gene increased by activation of Ob-Rb, and plays a key role in the regulation of leptin signalling by feedback inhibition of the leptin receptor (Banks et al., 2000; Bjørbæk et al., 2000). The level of SOCS-3 expression is a crucial determinant of leptin sensitivity. In Siberian hamsters (*Phodopus sungorus*) (Tups et al., 2006) and field voles (*Microtus agrestis*) (Król et al., 2006), photoperiod-induced modulation in leptin sensitivity is associated with changes in SOCS-3 gene expression. In Brandt's voles, SOCS-3 mRNA has been shown to increase significantly during pregnancy (Tang et al., 2008).

The central actions of leptin are partly mediated by inhibiting the orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP) neurons while stimulating the anorexigenic pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) neurons (Ahima et al., 2000; Schwartz et al., 2000). In lactating mice, NPY and AgRP increased and POMC and CART decreased (Smith, 1993; Chen et al., 1999; Xiao et al., 2005). It has been suggested that changes in neuropeptide expression in the arcuate nucleus are important in mediating the sustained hyperphagia associated with lactation (Smith and Grove, 2002; Xiao et al., 2005). However, there are few studies in wild species about the central mechanism of hyperphagia in lactation.

Brandt's voles [*Lasiopodomys brandtii* (Radde 1861)], well studied in ecological physiology in our laboratory, are typical steppe herbivores that mainly inhabit the Inner Mongolia grasslands of China, Mongolia and the region of Baikal in Russia, where mean annual temperature is 0–4°C. It has been reported that Brandt's voles are in a state of hyperphagia during lactation that is coupled with low serum leptin levels (Liu et al., 2003; Li and Wang, 2005a; Zhang and Wang, 2007). Serum leptin was negatively correlated with gross energy intake during lactation, suggesting that serum leptin is involved in the regulation of hyperphagia during lactation in Brandt's voles (Zhang and Wang, 2007). Interestingly, the serum leptin level was significantly higher during pregnancy and lower during lactation, although both phases are associated with hyperphagia in Brandt's voles (Zhang and Wang, 2007; Tang et al., 2008). Our previous results showed that hyperphagia is associated with elevated SOCS-3 and reduced POMC expression in pregnant Brandt's voles (Tang et al., 2008). Decreased leptin sensitivity has been considered as one cause of hyperphagia during pregnancy (Anderson et al., 2006; Ladyman, 2008). However, there is still a lack of studies on hypothalamic leptin sensitivity during lactation. We hypothesized that both the low circulating leptin level and the impaired hypothalamic leptin sensitivity contribute to the hyperphagia during lactation in Brandt's voles. To test this hypothesis, we examined the serum leptin level and SCOS-3 and Ob-Rb expression during different phases of lactation and analyzed their relationships with food intake in Brandt's voles. Additionally, we verified whether alterations of NPY, AgRP, POMC and CART gene expression in the hypothalamus can be involved in sustaining the hyperphagia during lactation in Brandt's voles.

MATERIALS AND METHODS
Animal and experimental protocol
The Brandt's voles used in the present study (90–120days old) were the offspring of the voles trapped in the Inner Mongolian Grasslands in May 1999 and raised at the Institute of Zoology, Chinese Academy of Sciences. Virgin voles were housed individually after 60days old in plastic cages (30×15×20cm) with sawdust and a little cotton as bedding. The voles were kept at 23±1°C under a 16h:8h light:dark photoperiod (lights on at 04:00h). Voles were fed commercial rabbit pellets (Beijing KeAo Feed Co., Beijing, China) and water was provided *ad libitum*. Seven females were randomly selected as the non-lactating group (NL, N=7). The other females were paired with males for 1 week to allow mating, and then the males were removed. The day of parturition was designated as day 0. Those females with a litter size of six to eight pups were selected and randomly assigned to the following groups: day 5 of lactation (L5, N=7), day 17 of lactation (L17, N=7) and day 27 of lactation (L27, N=7). The pups from the L27 group were weaned on day 20 of lactation. Dams were killed by puncture of the posterior vena cava on lactation days 5, 17 and 27, respectively; blood, tissue samples and brain samples were taken quickly for measurement of physiological parameters. Body mass and food intake were monitored on the day before the voles were killed. All animal procedures were licensed under the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences.

Food intake
Daily food intake was measured daily in metabolic cages as described previously (Liu et al., 2002; Song and Wang, 2003). During each test, weighed food was provided and water was provided *ad libitum*. Food residues and feces were collected, oven-dried at 60°C to constant mass and separated manually.

Body composition
The entire gastrointestinal tract was removed and the eviscerated carcass was weighed and then dried to constant weight at 60°C for determination of dry carcass mass. The difference between the wet carcass and dry carcass was the water mass of carcass. Body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus. Percent body fat content was determined as the body fat divided by wet carcass then multiplied by 100%.

Serum leptin assay
All dams were killed between 09:00 and 11:00h by puncture of the posterior vena cava. Blood was kept on ice for 30min before being centrifuged at 4000g for 30min at 4°C, and serum was sampled and stored at –75°C. Serum leptin levels were measured by radioimmunoassay (RIA) using the [125]I Multi-species Kit (Linco Research Inc., St Charles, MO, USA) in a single RIA (Li and Wang, 2005b). The lowest level of leptin that can be detected by this assay was 1.0ngml^{-1}. Inter- and intra-assay variability for leptin RIA was <3.6 and 8.7%, respectively.

Gene expression of hypothalamic Ob-Rb, SOCS-3 and neuropeptides
Gene expression of hypothalamic Ob-Rb, SOCS-3 and neuropeptides were measured as described previously (Tang et al., 2008).

Primer design
To amplify partial cDNA fragments of Brandt's vole NPY, AgRP, POMC, CART and β-actin, primers were designed based on the conserved regions of rat and mouse sequences available from GenBank. The fragments of Brandt's vole NPY, AgRP, POMC, CART and β-actin were attained by reverse transcription (RT)-PCR and sequenced. Homology analysis proved that these fragments of Brandt's voles came from the target genes (Tang et al., 2008). In the next step, primers for real-time PCR (Table 1) were designed from the fragments of Brandt's voles.

Total RNA isolation and cDNA synthesis
Total RNA was isolated from hypothalamus using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and treated by RNase-free DNase I (Promega, Madison, WI, USA) to remove any possible DNA residue; RNA integrity was identified by electrophoresis. Two micrograms of total RNA was transcribed using RT reagents (Fementas, Vilnius, Lithuania) in a 20μl reaction volume: 2μg total

Table 1. Primers used for real-time reverse transcription PCR

Primer	Oligonunucleotide sequence (5′–3′)	Product size (bp)
Ob-Rb (forward)	CTG AGA GGG GTT CTC TTT GT	147
Ob-Rb (reverse)	TCT TGC TCA TCC TCC GTT TC	
SOCS3 (forward)	AGA AGA TTC CGC TGG TAC TG	114
SOCS (reverse)	GCT GGG TCA CTT TCT CAT AG G	
NPY (forward)	TCG CTC TGT CCC TGC TCG TGT G	116
NPY (reverse)	TCT CTT GCC GTA TCT CTG CCT GGT G	
AgRP (forward)	GCC CTG TTC CCA GAG TTC CC	114
AgRP (reverse)	ATC TAG GAC CTC CGC CAA AGC	
POMC (forward)	AAG ATG GGC TCT ACG GGA TG	134
POMC (reverse)	GTT CTT GAC GAT GGC GTT CT	
CART (forward)	TGG AAC CTG GCT TTA GCA AC	145
CART (reverse)	TAC TCT GCA CAT GCC GAC AC	
β-actin (forward)	TTG TGC GTG ACA TCA AAG AG	200
β-actin (reverse)	ATG CCA GAA GAT TCC ATA CC	

Table 2. Body composition of Brandt's voles in different phases of lactation

	NL	L5	L17	L27	P
Sample size	7	7	7	7	
Body mass (g)	44.2±1.4[c]	60.6±1.9[a]	54.6±1.9[a,b]	50.3±1.2[b,c]	<0.01
Wet carcass (g)	28.9±1.5[b]	36.2±1.2[a]	32.7±0.7[a,b]	32.0±0.6[b]	<0.01
Dry carcass (g)	13.6±1.3	14.3±0.7	12.6±0.7	12.3±0.4	ns
Water mass of carcass (g)	15.3±0.4[b]	21.9±1.0[a]	20.0±0.5[a]	19.7±0.5[a]	<0.01
Body fat (g)	7.0±1.1	6.6±0.6	5.3±0.6	4.7±0.3	ns
Body fat content (%)	23.5±2.2[a]	18.3±1.4[a,b]	16.1±1.7[b]	14.7±1.1[b]	<0.01

L5, day five of lactation; L17, day 17 of lactation; L27, day 27 of lactation; NL, non-lactating group.
Values in a row with the same superscript do not differ significantly at $P<0.05$, as determined by one-way ANOVA and Tukey's honestly significant difference *post hoc* tests. ns, not significant.
Values are means ± s.e.m.

RNA, 1 μl (0.5 μg μl^{-1}) oligo(dt)$_{18}$ primer and DEPC-treated water to 12 μl. The mixture was incubated at 70°C for 5 min, chilled on ice and collected drops by brief centrifugation. The tube was placed on ice and the following components were added in order: 4 μl 5× reaction buffer, 1 μl (20 u μl^{-1}) Ribolock^{TM} Ribonuclease inhibitor and 2 μl 10 mmol l^{-1} dNTP mix. The mixture was incubated at 37°C for 5 min. Then 1 μl RevertAid^{TM} M-MuLV Reverse Transcriptase (200 u μl^{-1}) was added. The mixture was incubated at 42°C for 60 min. The reaction was stopped by heating at 70°C for 10 min then chilled on ice.

Real-time RT-PCR amplification
Real-time RT PCR amplification was carried out at a final 12.5 μl volume, containing 6.25 μl SYBR Premix EX Tag^{TM} (TaKaRa Biotechnology Co. Ltd, Dalian, Liaoning, China), 0.25 μl Rox, 0.25 μl (5 μmol l^{-1}) of each primer and 1 μl cDNA. Each PCR amplification was performed in triplicate wells including no-template controls for each gene, using the following conditions: 10 s at 95°C, followed by 40 cycles consisting of 5 s at 95°C, 20 s at 60°C and 20 s at 72°C. The fluorescence signals were read and collected at 72°C for each cycle. The real-time PCR was carried out on a MX3000P Real-Time PCR Detection System (Stratagene, La Jolla, CA, USA). Melting curve analysis showed a single PCR product after amplification of four hypothalamic genes and β-actin, and ending products of PCR were further confirmed by DNA sequencing. PCR amplicons were not generated from the no-template controls. We constructed standard curves for each gene *via* serial dilutions of cDNA (onefold to 32-fold dilutions). Analysis of standard curves between target genes and β-actin showed that they had similar amplification efficiencies (0.95), which ensures the validity of comparative relative quantity method. The quantities of gene mRNA were normalized with β-actin mRNA to compensate for possible variations from mRNA extraction and reverse transcription, and then were expressed as relative units (RU).

Statistical analyses
Data were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene's tests, respectively. Group differences in body mass, food intake, body composition, leptin levels, leptin sensitivity parameters and neuropeptides were analysed by one-way ANOVA. Pearson's correlation was performed to determine the correlation between food intake and leptin or neuropeptides expression. Values are expressed as means ± s.e.m., and $P<0.05$ was considered to be statistically significant.

RESULTS
Body mass and body composition
Body mass was significantly higher on lactation day 5 than in the non-lactating group ($P<0.001$), and it decreased on lactation day 17 and reached the level of non-lactating group on lactation day 27 ($P>0.05$; Table 2). Body fat on lactation days 17 and 27 was significantly lower than that of the non-lactating group ($P<0.05$; Table 2). Lactating females had higher carcass water masses than non-lactating females ($P<0.01$; Table 2).

Food intake and serum leptin levels
Compared with non-lactating females, lactating females significantly increased their food intake on lactation day 5 by 67% ($P<0.05$) and by 105% on lactation day 17 ($P<0.001$). After weaning, food intake in lactating females was restored to the level of non-lactating females ($P>0.05$). Even after adjustment for body mass, the difference in daily food intake per gram body mass remained significant among groups (Fig. 1A). Serum leptin levels were significantly lower in lactating voles compared with non-lactating voles ($F_{3,24}=22.756$, $P<0.001$; Fig. 1B) and no significant differences were found between different stages of lactation ($P>0.05$; Fig. 1B). Pearson correlation analysis indicated that serum leptin levels were negatively correlated with food intake ($r=-0.404$, $P<0.05$; Fig. 1C) and positively correlated with body fat percentage ($r=0.511$, $P<0.01$).

Hypothalamic Ob-Rb and SOCS-3 mRNA expression
Compared with non-lactating females, the mRNA expression of hypothalamic Ob-Rb ($F_{3,24}=0.421$, $P>0.05$; Fig. 2A) and SOCS-3 ($F_{3,24}=0.410$, $P>0.05$; Fig. 2B) was unchanged during lactation. There was no relationship between serum leptin and Ob-Rb ($r=0.249$, $N=28$, $P>0.05$) or SOCS-3 ($r=-0.279$, $N=28$, $P>0.05$) mRNA levels.

Hypothalamic NPY, AgRP, POMC and CART mRNA expression
The mRNA expression of the hypothalamic orexigenic neuropeptides NPY ($F_{3,24}=6.817$, $P<0.05$; Fig. 3A) and AgRP ($F_{3,24}=4.822$, $P<0.05$; Fig. 3B) was significantly increased during lactation whereas that of the anorexigenic neuropeptide POMC ($F_{3,24}=6.052$, $P<0.05$; Fig. 3C) was significantly decreased. NPY mRNA increased significantly by 125 and 143% on lactation days 5 and 17, respectively, compared with non-lactating voles, and was restored to the level of non-lactating voles after weaning. On lactation day 17, AgRP mRNA expression increased significantly by 110% compared with non-lactating voles. POMC mRNA expression decreased significantly by 53 and 45% on lactation days 5 and 17, respectively, compared with non-lactating voles. In
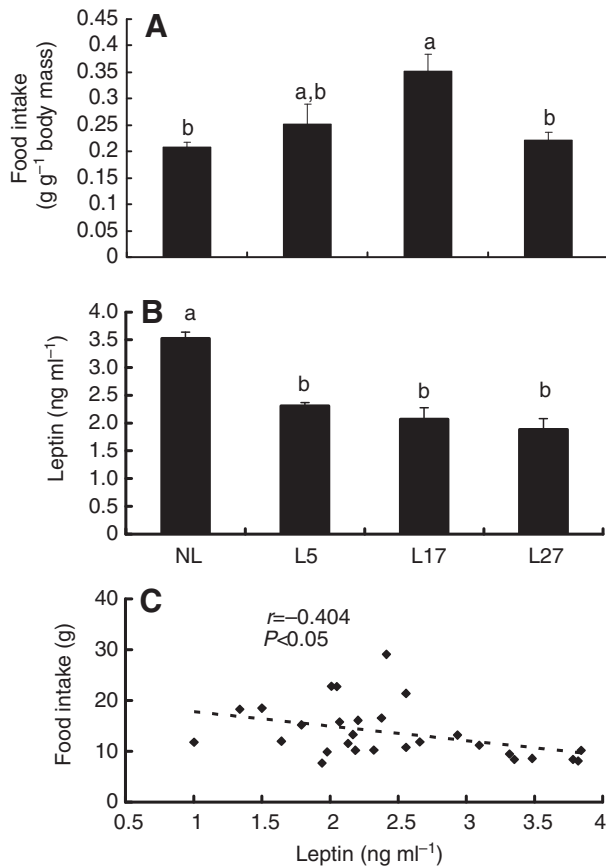
Fig. 1. Food intake (A) and leptin levels (B) in different phases of lactation in Brandt's voles. Values in a row with the same superscript do not differ significantly at *P*<0.05, as determined by one-way ANOVA and Tukey's honestly significant difference (HSD) *post hoc* tests. L5, lactation day 5; L17, lactation day 17; L27, lactation day 27; NL, non-lactating group. (C) Correlation of food intake with leptin (all groups were pooled for the correlation analysis).

Fig. 2. Hypothalamic Ob-Rb (A) and SOCS-3 (B) mRNA expression in different phases of lactation in Brandt's voles.

contrast, there were no significant changes in hypothalamic CART mRNA expression over the lactation period ($F_{3,24}=0.133$, $P>0.05$; Fig. 3D). Pearson correlation analysis indicated that food intake was positively correlated with NPY ($r=0.685$, $N=28$, $P<0.001$; Fig. 4A) and AgRP ($r=0.619$, $N=28$, $P<0.001$; Fig. 4B) mRNA expression and negatively correlated with POMC ($r=-0.489$, $N=28$, $P>0.01$; Fig. 4C) mRNA expression.

DISCUSSION

In the present study, lactating Brandt's voles increased their food intake markedly on day 5 (by 67%) and day 17 (by 105%) of lactation, and serum leptin levels decreased significantly during lactation. As such, serum leptin levels were negatively correlated with food intake. These data indicated that low serum leptin levels might play an important role in inducing hyperphagia during lactation. However, it can be seen from Fig. 1C that leptin explains only approximately 16% of the variance in food intake. Consistent with this idea, a leptin replacement experiment (Cui et al., 2011) showed that only approximately 16% of the increase in energy intake at peak lactation in the Brandt's voles was attributed to the suppression of leptin levels. These data therefore suggest that other factors may play roles in the hyperphagic response during lactation.

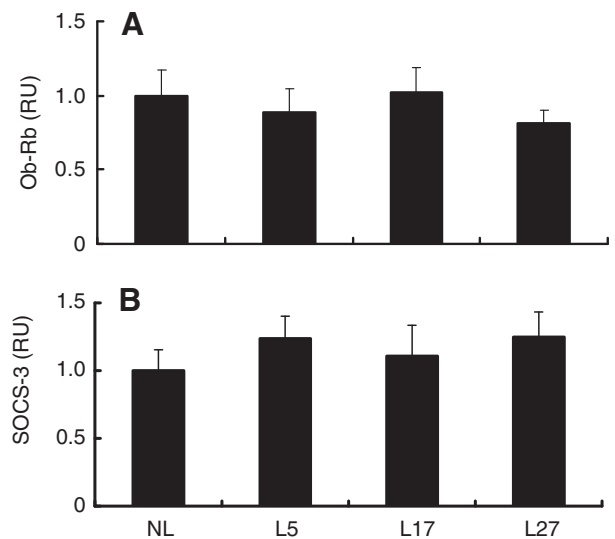Besides the low leptin concentration, change in leptin sensitivity is a potential factor of hyperphagia, as has been shown in diet-induced obese mice (Munzberg et al., 2005) and pregnant rats (Anderson et al., 2006; Ladyman, 2008). Ob-Rb was thought to be crucially involved in regulating leptin sensitivity in pregnant rats (Ladyman and Grattan, 2004). However, our results showed that hypothalamic Ob-Rb mRNA did not change during lactation in Brandt's voles. We have also found that leptin infusion did not cause the Ob-Rb mRNA change in cold-exposed Brandt's voles (Tang et al., 2009). Denis et al reported that Ob-Rb mRNA expression in lactating rats was lower during the light phase and higher during the dark phase than in non-lactating rats (Denis et al., 2003b). Decreased daytime Ob-Rb expression during lactation could lead to reduced hypothalamic sensitivity to leptin, and thus to increased daytime appetite in lactating rats (Denis et al., 2003b). Non-lactating rats have been shown to consume more than 80% of their food at night (Bruckdorfer et al., 1974). But Brandt's voles consume the same amount of food during day and night (Liu et al., 2007). Thus, the differences of Ob-Rb expression pattern during lactation between wild Brandt's voles and laboratory rats may be due to the different rhythms of feeding behavior or other selection forces.

SOCS-3 acts as a feedback inhibitor of leptin signaling in the hypothalamus and is thought to be another crucial factor that affects leptin sensitivity (Howard et al., 2004; Mori et al., 2004). It has been demonstrated that elevation of SOCS-3 mediated leptin resistance in diet-induced obese mice (Mori et al., 2004), age-related obese rats (Scarpace et al., 2002), long-photoperiod acclimated Siberian hamsters (Tups et al., 2004) and pregnant rats (Ladyman, 2008). In pregnant Brandt's voles, the hypothalamic SOCS-3 mRNA expression was elevated and suggested to be involved in leptin resistance (Tang et al., 2008). However, the present results showed that SOCS-3 mRNA expression in whole hypothalamus was unchanged, similar with our previous findings in cold-exposed Brandt's voles (Tang et al., 2009). In contrast, SOCS-3 expression in the arcuate nucleus was elevated during lactation in rats (Anderson et al., 2006). The reason for this difference is unclear and may be due to the species-specific differences or differences in the methodology used to measure mRNA expression. For lactating Brandt's voles in the present study, leptin levels decreased and food intake increased, and SOCS-3 mRNA was unchanged. This pattern
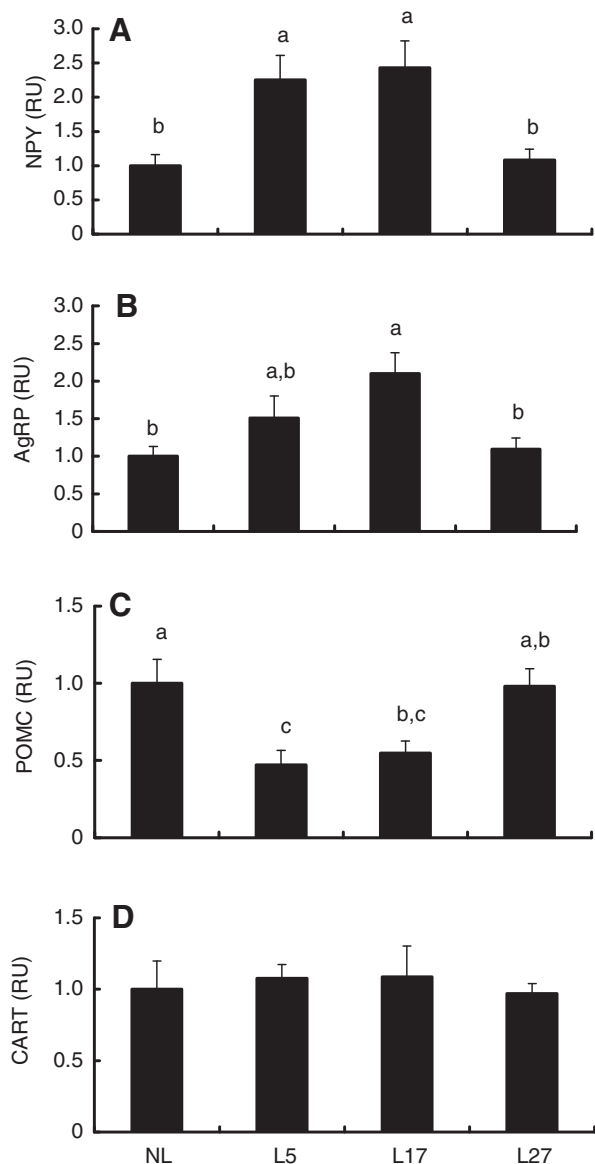
Fig. 3. Hypothalamic NPY (A), AgRP (B), POMC (C) and CART (D) mRNA expression in different phases of lactation in Brandt's voles. Values in a row with the same superscript do not differ significantly at *P*<0.05, as determined by one-way ANOVA and Tukey's HSD *post hoc* tests.

Fig. 4. Correlations of food intake with NPY (A), AgRP (B), POMC (C) and CART (D) mRNA expression in all groups in Brandt's voles.

is different from that in pregnant Brandt's voles, in which leptin levels, food intake and hypothalamic SOCS-3 mRNA increased (Tang et al., 2008). The cause of different patterns in SOCS-3 expression between lactation and pregnancy may be partly due to different leptin concentrations.

Many studies have shown that the effect of leptin on food intake was mediated by the hypothalamic neuropeptides NPY, AgRP, POMC and CART (reviewed by Ahima et al., 2000). In the present study, NPY and AgRP mRNA expression increased significantly during lactation whereas POMC mRNA expression decreased significantly. These results further support the roles of these peptides in the regulation of food intake. Food intake was positively correlated with NPY and AgRP mRNA expression but negatively correlated with POMC mRNA expression during lactation. These results indicate that NPY, AgRP and POMC were involved in regulation of food intake during lactation. Compared with the non-
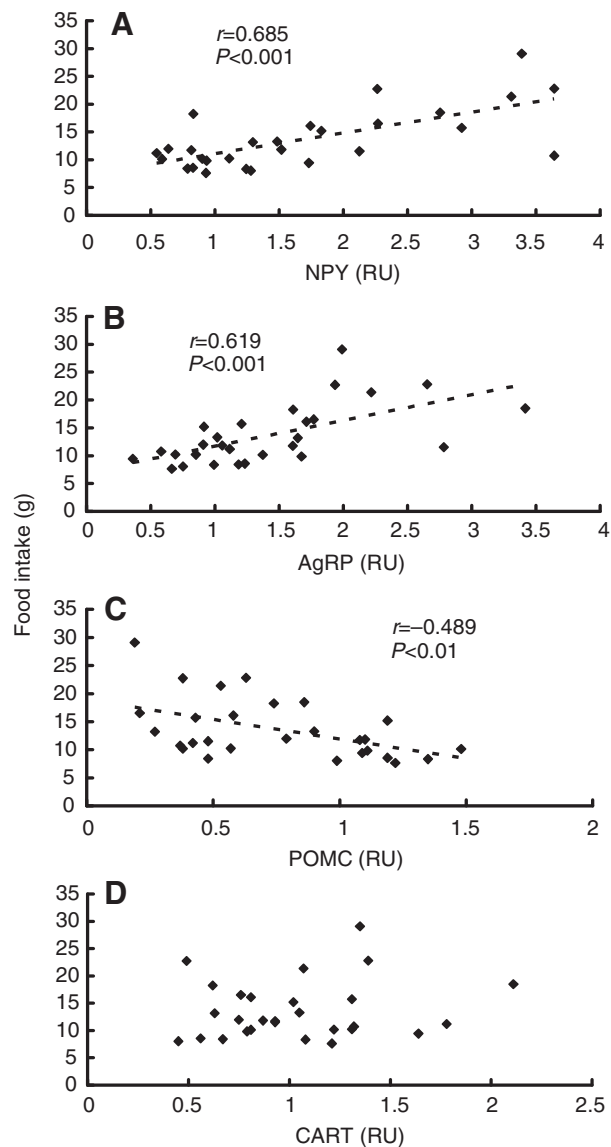
lactating females, serum leptin levels in lactating voles decreased significantly, coupled with higher NPY and AgRP expression and lower POMC expression. Recent results of the leptin replacement experiment in Brandt's voles show that leptin infusion significantly decreased the expression of NPY and AgRP and increased the expression of POMC (Cui et al., 2011). Results in rats have also indicated that leptin administration to lactating females reversed the upregulation of NPY and AgRP mRNA expression in the arcuate nucleus (Crowley et al., 2004). These data showed that leptin could be involved in regulating hypothalamic neuropeptides in small lactating female mammals. After weaning, leptin level was not restored to the level of non-reproductive females because of the low body fat content. Interestingly, although the leptin levels were unchanged after weaning, NPY, AgRP and POMC expression were restored to the level of non-reproductive females, coupled with the decrease in food intake. Further, it can be seen from Fig. 4 that NPY

explains approximately 47% and AgRP approximately 38% of the variance in food intake whereas leptin explains only approximately 16% of the variance in food intake (Fig. 1C). These data suggest that other neuronal pathways, apart from the classical leptin signaling pathway, such as prolactin and insulin, are involved in the regulation of these neuropeptides and hyerphagia during lactation (Crowley et al., 2004; Woodside, 2007).

In conclusion, serum leptin levels were decreased whereas hypothalamic Ob-Rb and SOCS-3 were unchanged during lactation in Brandt's voles, indicating that lactation-induced hyperphagia is associated with low leptin levels but not impaired leptin sensitivity. Results indicate that the hypothalamic neuropeptides NPY, AgRP and POMC are involved in mediating the roles of leptin in regulation of food intake during lactation.

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