Journal of Neuroendocrinology
From Molecular to Translational Neurobiology

Journal of Neuroendocrinology **20**, 1038–1044

ORIGINAL ARTICLE

Hypothalamic Suppressor-of-Cytokine-Signalling 3 mRNA is Elevated and Pro-Opiomelanocortin mRNA is Reduced During Pregnancy in Brandt's Voles (*Lasiopodomys brandtii*)

G.-B. Tang,*† J.-G. Cui*† and D.-H. Wang*

**State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Datun Lu, Chaoyang, Beijing, China.*

†Graduate School of the Chinese Academy of Sciences, Yuquan Lu, Beijing, China.

Journal of Neuroendocrinology

Leptin acts within the hypothalamus to diminish food intake. In Brandt's voles (*Lasiopodomys brandtii*), both circulating leptin levels and food intake are elevated during pregnancy, suggesting an ineffectiveness of leptin to reduce food intake. Diminished hypothalamic leptin receptors and impaired leptin signal transduction are characteristic of central leptin resistance. The present study aimed to determine whether these characteristic modulations of leptin sensitivity occurred in pregnant Brandt's voles. The mRNA expression of the long form of the leptin receptor (Ob-Rb), suppressor-of-cytokine-signalling 3 (SOCS3), 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 10 and day 18 of pregnancy. Compared to controls, there was no significant change in hypothalamic Ob-Rb mRNA during the pregnancy. SOCS3 mRNA was increased significantly by 68% on day 10% and 93% on day 18 of pregnancy compared to controls. Despite elevated leptin levels, POMC mRNA was decreased significantly by 60% on day 18 of pregnancy, whereas no differences were found in the mRNA expression of NPY, AgRP and CART in pregnant voles compared to controls. The elevation of SOCS3 mRNA together with disrupted leptin regulation of neuropeptides in the hypothalamus suggests that leptin resistance may develop in pregnant Brandt's voles.

Key words: *Lasiopodomys brandtii*, leptin resistance, neuropeptide, pregnancy.

doi: 10.1111/j.1365-2826.2008.01764.x

Correspondence to:
Dr De-Hua Wang, Institute of Zoology, Chinese Academy of Sciences, Datun Lu, Chaoyang, Beijing 100101, China (e-mail: wangdh@ioz.ac.cn).

During pregnancy, females undergo a range of physiological adaptations to meet the energy requirements of fetal development and to prepare for the subsequent lactation. These include significant increase in appetite and food intake (1, 2). Interestingly, leptin levels also increase during pregnancy. This increase is paradoxical because elevated leptin levels typically result in reduced food intake (3, 4). The combination of increased leptin levels at the same time as an increase in food intake strongly suggests that pregnancy involves a state of leptin resistance (5). However, the mechanisms underlying this pregnancy-induced leptin resistance are not well understood.

Inhibition of the intracellular long form of leptin receptor (Ob-Rb) signalling cascade is one of important mechanisms suggested to underpin leptin resistance (6). Among six leptin receptor isoforms, Ob-Rb is the only one with full intracellular signal transduction capacity (7). Reduced Ob-Rb expression in the hypothalamus was involved in the development of leptin resistance in pregnant rats (8–10). Suppressor-of-cytokine-signalling 3 (SOCS3) 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 (11, 12). It has been identified as a potential mediator of central leptin resistance in several animal models (13–15).

Leptin's central effects are initiated by Ob-Rb activation and then mediated through a hierarchy of both anorectic and orexigenic neuropeptidergic neurones in specific sites within the hypothalamus (16–18). Within the arcuate nucleus (ARC), anorectic pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) are colocalised in a distinct subset of arcuate neurones, while the orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP) are colocalised in a distinct but adjacent subset of ARC neurones (17). Both of these two types of ARC

neurones express the Ob-Rb (19). It has been demonstrated that leptin regulates these two types of neurones in a reciprocal manner by inhibiting AgRP/NPY neurones while stimulating POMC/CART neurones (20). Previous studies suggested that deficient activation of these leptin-responsive neuropeptides by leptin may be one element of pregnancy-induced leptin resistance (21–23).

Brandt's voles, a previously well-described seasonal animal (24, 25), exhibit hyperphagia accompanied by elevated leptin levels during pregnancy (26, 27), suggesting a state of leptin resistance. The central leptin resistance is characterised by diminished hypothalamic leptin receptors and impaired leptin signal transduction (9, 23, 28). Therefore, we examined the gene expression of Ob-Rb, SOCS3, NPY, AgRP, POMC and CART in the hypothalamus to identify whether characteristic changes of central leptin resistance occurred in pregnant Brandt's voles.

Materials and methods

Animals and experimental protocols

All animal procedures were licensed under the Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences. Virgin female Brandt's voles (100–120 days old) were from our laboratory colony, and maintained at 23 ± 1 °C under a 16 : 8 h light/dark cycle (lights on 04.00 h). Voles were weaned at 18–20 days of age and kept in single-sex groups of three to four animals in plastic cages (30 × 15 × 20 cm) that contained sawdust bedding. Commercial rabbit pellets (Beijing KeAo Feed Co., Beijing, China) and water were available *ad libitum*. The voles were individually-housed for 1 month prior to the experiment. Virgin females at oestrous were mated overnight, and the next morning on which a vaginal plug was present was designated day 0 of pregnancy.

To continually monitor the changes in body weight and food intake during pregnancy in Brandt's voles, we measured body weight and food intake of pregnant (n = 8) and nonpregnant (n = 8) voles at 2 days intervals throughout pregnancy. To monitor the changes in serum leptin levels, body fat mass, Ob-Rb, SOCS3, NPY, AgRP, POMC and CART during 18 days of pregnancy in Brandt's voles, four groups of voles were sacrificed between 09.00 and 11.00 h on dioestrous (n = 8), day 5 (n = 8), day 10 (n = 8) and day 18 (n = 8) of pregnancy. Trunk blood was collected and centrifuged at 4000 *g* for 30 min after a 30-min interval at 4 °C. Serum was collected and stored at −80 °C for leptin determination. As previously described (29), a slice of brain tissue was cut between the optic chiasm and the mammillary bodies, and the hypothalamus was dissected by a horizontal cut immediately below the anterior commissure and vertical cuts through the edge of the septum and perihypothalamic sulcus. The hypothalamus was frozen in liquid nitrogen immediately and stored at −80 °C until subsequent analysis.

Measurement of body weight and food intake

Body weight and food intake were measured at 09.00 h. During each test, voles were weighed and housed individually in metabolic cages, where food and water were provided *ad libitum*. Two days later, the animals were re-weighed and all food residues and faeces were collected and oven-dried at 60 °C to constant weight. Subsequently, they were separated manually.

Measurement of body fat mass

After dissection of the hypothalamus and brown adipose tissue, internal organs were removed and the eviscerated carcass was weighed and dried in an oven at 60 °C to constant weight. Then, the dry carcass mass (W1) was weighed and recorded. After grinding the dry carcass in a mill and mixing it completely, 1 g of sample (W2) was weighed to an accuracy of ±1 mg into a thimble (W3). Body fat extraction was performed in a Soxtec Fat Extraction Systems (Soxtex Avanti 2050, FOSS, Hogänäs, Sweden) with petroleun ether. Subsequently, the thimble contained residual sample was dried in an oven at 60 °C to a constant weight and weighed it (W4). Finally, the carcass fat mass was calculated using the formula:

$$\text{Carcass fat mass(mg)} = (W3 + W2 - W4) \times W1/W2$$

Serum leptin assays

Serum leptin levels were measured by radioimmunoassay (RIA) with a ^{125}I multi-species kit (Cat. No. XL-85K, Linco Research Inc., St Charles, MO, USA), which has been validated previously in Brandt's voles (24). The lower and upper limits of the assay kit were 1 and 50 ng/mL and the inter- and intra-assay variations were < 3.6% and 8.7%, respectively. Serum leptin values were determined in a single RIA and expressed as nanograms of leptin per milliliter.

Real-time reverse transcription-polymerase chain reaction (RT-PCR) assay of hypothalamic gene expression of Ob-Rb, SOCS3, NPY, AgRP, POMC and CART

Primer design

In the first step, according to the recorded gene sequences of rats or mice in genbank, we designed the primers of Ob-Rb, SOCS3, NPY, AgRP, POMC, CART and β-actin and obtained part gene sequences of their homologous genes in Brandt's voles. Homology analysis proved that these nucleotide fragments all come from the target genes (Table 1). In the next step, species-specific primers (Table 2) set for these genes were designed based on these cloned sequences.

Total RNA isolation and cDNA synthesis

Total RNA was isolated using TRIzol Reagent (Cat. No. 15596-026, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. For unknown reasons, total RNA was not detected in two hypothalamus samples after TRIzol extraction. Therefore, we excluded these samples. To remove any

Table 1. Homology Analysis of Targeted Gene Fragments Derived From Brandt's Voles.

Targeted gene	Product size (bp)	Homology with rat (%)	Homology with mouse (%)
Ob-Rb	905	87	88
SOCS3	685	89	87
NPY	423	92	91
AgRP	340	86	89
POMC	754	93	87
CART	561	95	87
β-actin	759	94	94

Ob-Rb, long form of the leptin receptor; SOCS3, suppressor-of-cytokine-signalling; NPY, neuropeptide Y; AgRP, agouti-related protein; POMC, pro-opiomelanocortin; CART; cocaine- and amphetamine-regulated transcript.

Table 2. Gene-Specific Primers Used for Real-Time RT-PCR.

Primers	Oligonuncleotide sequence (5′ to 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
SOCS3 (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	

Ob-Rb, long form of the leptin receptor; SOCS3, suppressor-of-cytokine-signalling; NPY, neuropeptide Y; AgRP, agouti-related protein; POMC, pro-opiomelanocortin; CART; cocaine- and amphetamine-regulated transcript.

contaminating DNA, RNA samples were treated with DNase I (Cat. No. M6101, Promega, USA) for 30 min at 37 °C followed by another cycle of TRIzol extraction to eliminate residual DNase I. The A260/280 ratio of total RNA was found to be approximately 1.9 using Beckman Coulter DU 800 spectrophotometer (Beckman, Fullerton, CA, USA). An equal amount (3 μg) of total RNA was transcribed into first strand cDNA for each sample using reverse transcription kit (Cat. No.1622, Fermentas, Vilnius, The Republic of Lithuania) according to the manufacturer's instruction.

Real-time RT-PCR

The cDNA was subjected to real-time PCR amplification using the SYBR Green I qPCR kit (Cat. NO. DRR041D, TaKaRa, Shiga, Japan) in the Mx3000P quantitative PCR system (Stratagene, La Jolla, CA, USA). Real-time RT-PCR was carried out in 12.5 μL reaction agent comprised of 6.25 μL 2 × SYBR® Premix EX Taq™master mix, 1 μL cDNA templates and 0.2 μmoL/L primers. Each sample was analysed in duplicate. Thermal cycling conditions were: 95 °C for 10 s, 40 cycles of 95 °C for 5 s, 60 °C for 20 s, and 72 °C for 20 s. Melting curve analysis showed a single PCR product after amplification of five hypothalamic genes and β-actin, and ending-products of PCR were further confirmed by DNA sequencing. We constructed standard curves for each gene via serial dilutions of cDNA (1–2^6-fold dilutions). Analysis of standard curves between target genes and β-actin showed that they had similar amplification efficiency, which ensures the validity of comparative quantity method. The data derived from Mx3000P quantitative software were expressed as relative amounts, which were calculated by normalising the amount of target gene mRNA levels to the amount of β-actin mRNA levels. No amplification was detected in absence of template or in the no RT control.

Statistical analysis

Data were analysed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for assumptions of normality of variance using the Kolmogorov–Smirnov tests. Non-normally distributed data of hypothalamic gene expression underwent arcsine square-root transformation. The significance of temporal changes in body weight and food intake in pregnant and dioestrous voles were assessed by repeated measures ANOVA, followed by LSD post-hoc test. Group differences in body weight and food intake were assessed by an independent-samples t-test. Differences among groups in body fat mass, serum leptin levels, the mRNA levels of hypothalamic Ob-Rb, NPY, AgRP, POMC and CART were assessed by one-way ANOVA, followed by the Tukey post-hoc test. Pearson correlation analyses were used to detect possible associations of serum leptin levels with body fat mass. Spearman correlation was employed to examine the correlation between serum leptin levels and SOCS3 mRNA. Because serum leptin levels were significantly correlated with SOCS3 mRNA, group differences in SOCS3 mRNA were tested by ANCOVA with serum leptin level as a covariate, followed by the LSD comparison between the means. Data are expressed as the mean ± SE. $P < 0.05$ was considered statistically significant.

Results

Body weight changes

Body weight was increased significantly over time within both nonpregnant ($F_{9,63} = 3.59$, $P = 0.001$; Fig. 1) and pregnant voles ($F_{9,63} = 72.23$, $P < 0.001$; Fig. 1). For the eight pregnant voles, body weight showed no significant changes ($P > 0.05$) at day 2 of pregnancy. From day 4 to day 18, body weight in pregnant Brandt's voles was increased significantly ($P < 0.05$). There was no difference in body weight between nonpregnant and pregnant voles prior to the experiment ($t = -1.23$, d.f. = 14, $P > 0.05$; Fig. 1). On day 4, body weight in the pregnant voles became higher than nonpregnant voles ($t = 2.25$, d.f. = 14, $P < 0.05$; Fig. 1). On day 18, body weight in the pregnant voles was increased by 57% ($t = 6.55$, d.f. = 14, $P < 0.01$; Fig. 1), compared to nonpregnant voles.

Food intake changes

There were significant changes in food intake within both nonpregnant ($F_{8,56} = 2.48$, $P < 0.05$; Fig. 2) and pregnant voles ($F_{8,56} = 5.27$, $P < 0.001$; Fig. 2) over time. The eight pregnant voles continually increased their daily food intake from 10.8 ± 1.0 g on day 0 to 12.9 ± 1.3 g on day 18. For the eight nonpregnant voles,

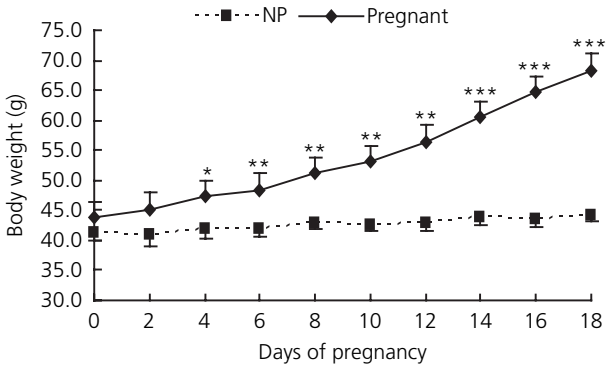

Fig. 1. Body weight in nonpregnant (NP; n = 8) and pregnant Brandt's voles (n = 8) during 18 days of pregnancy. Data are expressed as the mean ± SEM. *Significant with respect to nonpregnant values ($P < 0.05$). **Significant with respect to nonpregnant values ($P < 0.01$). ***Significant with respect to nonpregnant values ($P < 0.001$).

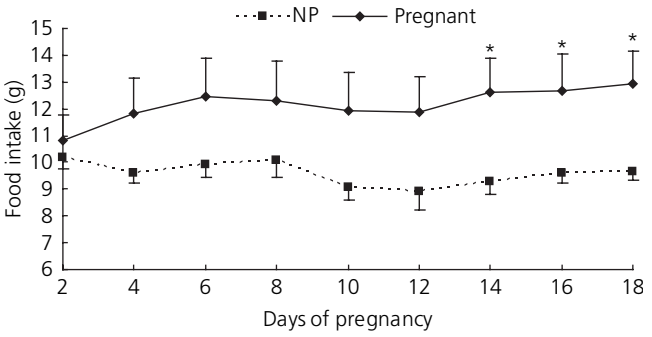

Fig. 2. Food intake in nonpregnant (NP; n = 8) and pregnant Brandt's voles (n = 8) during 18 days of pregnancy. Data are expressed as the mean ± SEM. *Significant with respect to nonpregnant values (P < 0.05).

daily food intake averaged 10.2 ± 0.4 g on day 0 and showed a decrease throughout the experiment, ending at 9.7 ± 0.3 g on day 18 (Fig. 2). Initially, there were no significant differences in food intake between pregnant and nonpregnant voles (nonpregnant: 10.2 ± 0.4 g, pregnant: 10.8 ± 1.0 g, t = 0.57, d.f. = 14, P > 0.05; Fig. 2). On day 14, day 16 and day 18, food intake in the pregnant voles was increased significantly by 30% (t = −2.49, d.f. = 14, P < 0.05; Fig. 2), 32% (t = −2.14, d.f. = 14, P = 0.05; Fig. 2) and 33% (t = −2.51, d.f. = 14, P < 0.05; Fig. 2) respectively, compared to nonpregnant voles.

Body fat mass and serum leptin levels

Pregnant voles showed significant increase in body fat mass during 18 days of pregnancy ($F_{3,31}$ = 7.497, P < 0.01; Fig. 3A). On day 18, body fat mass in pregnant group was significantly increased by 58%, compared to controls.

Serum leptin levels were significantly higher in pregnant voles compared to nonpregnant voles ($F_{3,31}$ = 4.80, P < 0.01; Fig. 3B). On day 18 of pregnancy, serum leptin level was increased significantly by 82% compared to controls. Correlation analysis indicated that serum leptin levels were positively with body fat mass (r = 0.628, P < 0.001; Fig. 3C).

Hypothalamic Ob-Rb and SOCS3 mRNA expression

There were no significant changes in the mRNA expression of hypothalamic Ob-Rb between the control and pregnant voles ($F_{3,29}$ = 1.36, P > 0.05; Fig. 4). However, it showed a marked decrease (48%) on day 10 of pregnancy compared to controls, although the difference did not get to statistically significant. There was also no significant correlation between serum leptin and Ob-Rb mRNA levels (r = 0.334, n = 30, P > 0.05).

Serum leptin levels were positively correlated with SOCS3 mRNA levels (r = 0.453, n = 30, P < 0.05; Fig. 5A). When the effect of leptin was removed, hypothalamic SOCS3 mRNA levels were increased during pregnancy ($F_{3,29}$ = 5.984, P < 0.01; Fig. 5B).

On day 10 and day 18 of pregnancy, SOCS3 mRNA in pregnant voles was increased significantly by 68% and 93%, respectively, compared to controls.

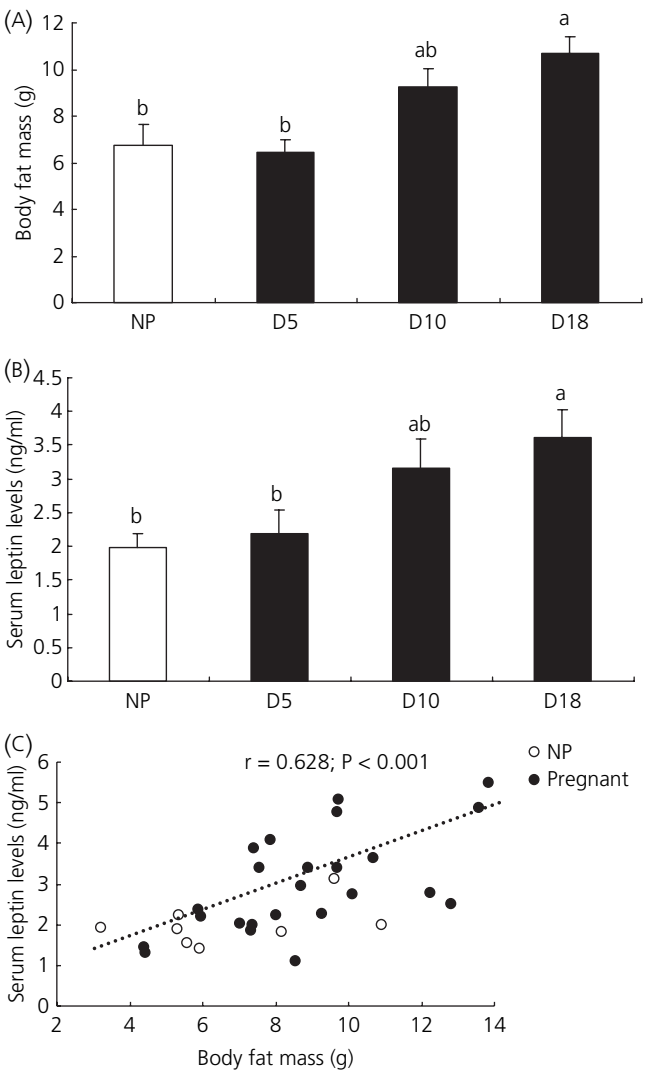

Fig. 3. Effects of pregnancy on body fat mass (A) and serum leptin levels (B) in Brandt's voles. Correlation between serum leptin levels and body fat mass (C). Data are expressed as the mean ± SEM. Statistically significant difference (P < 0.05) are marked with different letters corresponding to the groups. NP, nonpregnant dioestrous group; D5, day 5 of pregnancy group; D10, day 10 of pregnancy group; D18, day 18 of pregnancy group.

Hypothalamic NPY, AgRP, POMC and CART mRNA expression

There were no significant changes in the mRNA expression of hypothalamic NPY ($F_{3,29}$ = 0.10, P > 0.05), AgRP ($F_{3,29}$ = 0.29, P > 0.05), and CART ($F_{3,29}$ = 0.30, P > 0.05; Fig. 6B) between the control and pregnant voles. In contrast, hypothalamic POMC mRNA was significantly decreased during the pregnancy ($F_{3,29}$ = 4.62, P = 0.01; Fig. 6A). On day 18 of pregnancy, POMC mRNA was decreased significantly by 60% compared to controls.

Discussion

During pregnancy, Brandt's voles showed elevated leptin levels, as has been previously reported in mice (30), rats (31), sheep (32),

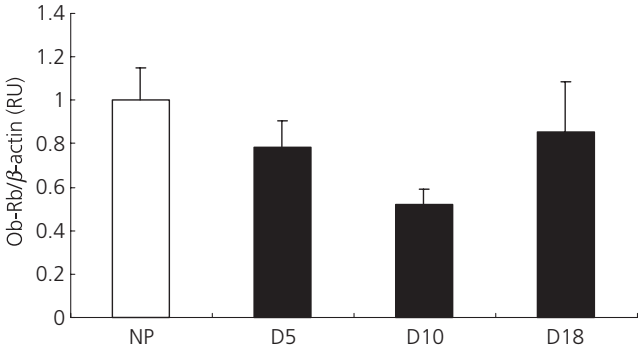

Fig. 4. Effects of pregnancy on the long form of the leptin receptor (Ob-Rb) gene expression in the hypothalamus in Brandt's voles. The graph shows the relative abundance of mRNA, expressed as a ration to β-actin mRNA. Data are expressed as the mean ± SEM. NP, nonpregnant dioestrous group; D5, day 5 of pregnancy group; D10, day 10 of pregnancy group; D18, day 18 of pregnancy group.

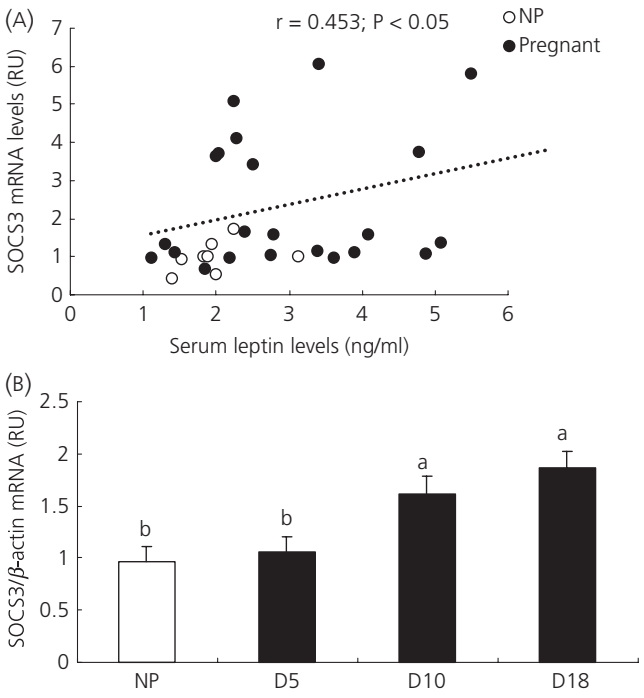

Fig. 5. (A) Correlation between suppressor-of-cytokine-signalling (SOCS3) mRNA and serum leptin levels. (B) Effects of pregnancy on SOCS3 gene expression in the hypothalamus in Brandt's voles. The graph shows the relative abundance of mRNA, expressed as a ration to β-actin mRNA. Data are expressed as the mean ± SEM. Statistically significant difference ($P < 0.05$) are marked with different letters corresponding to the groups. NP, nonpregnant dioestrous group; D5, day 5 of pregnancy group; D10, day 10 of pregnancy group; D18, day 18 of pregnancy group.

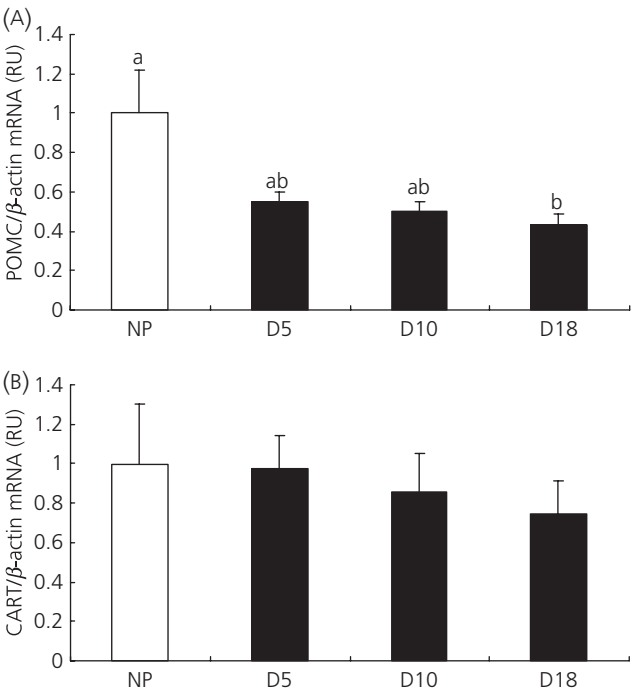

Fig. 6. Effects of pregnancy on (A) pro-opiomelanocortin (POMC) and (B) cocaine- and amphetamine-regulated transcript (CART) gene expression in the hypothalamus in Brandt's voles. The graph shows the relative abundance of mRNA, expressed as a ration to β-actin mRNA. Data are expressed as the mean ± SEM. Statistically significant difference ($P < 0.05$) are marked with different letters corresponding to the groups. NP, nonpregnant dioestrous group; D5, day 5 of pregnancy group; D10, day 10 of pregnancy group; D18, day 18 of pregnancy group.

baboons (33) and humans (34). Our data showed that serum leptin levels were positively correlated with body fat mass, suggesting that increased body fat contributes to the elevation of leptin. Proposed physiological roles for leptin in pregnancy include the regulation of conceptus development, fetal/placental angiogenesis, embryonic haematopoiesis and hormone biosynthesis within the maternal–fetoplacental unit (35). In addition, several studies suggested that a state of leptin resistance during pregnancy would allow the mother to increase food intake, thus ensuring adequate energy supplies to meet the high energy demands of the subsequent lactation (28, 36, 37). However, whether the elevation of leptin during pregnancy is the cause or consequence of leptin resistance remains to be determined.

The reduction of Ob-Rb contributes to the development of leptin resistance during pregnancy in rats (9). In the present study, hypothalamic Ob-Rb mRNA decreased markedly (48%) on day 10 of pregnancy compared to controls, although these effects fell short of statistical significance. Limited sample size in the present study may be the possible cause. Interestingly, Ob-Rb mRNA level was similar between late pregnant and control voles. The changes in Ob-Rb mRNA in the present study are inconsistent with a previous study showing that there was a significant reduction of Ob-Rb mRNA in the ventromedial nucleus throughout pregnancy in rats (9). However, the methodology employed in the present study does not allow us to distinguish mRNA expression in specific hypothalamic nuclei, and it is possible that differences of Ob-Rb gene expression in specific subsets of neurones could be masked in the whole hypothalamus.

SOCS3 is another critical determinant of leptin sensitivity (38, 39). The elevation of SOCS3 was shown to mediate central leptin resistance in several leptin resistant animal models, such as long photoperiod acclimated Siberian hamsters (*Phodopus sungorus*) (14) and field voles (*Microtus agrestis*) (15), A^y/a mice (13), DIO C57BL/6J mice (40) and age-related obese rats (41).

The present results showed that hypothalamic SOCS3 mRNA increased as pregnancy progressed. SOCS3 mRNA remained unchanged on day 5 of pregnancy compared to controls. At this point, serum leptin levels also remained stable, suggesting that leptin signalling might be unchanged on day 5 of pregnancy. Interestingly, hypothalamic SOCS3 mRNA increased significantly on days 10 and 18 of pregnancy compared to controls. Simultaneous increases in food intake and serum leptin level on day 18 of pregnancy suggest the development of leptin resistance around late pregnancy. Together with the important role for SOCS3 in inhibiting leptin signalling, our data suggest that the elevation of SOCS3 mRNA may be involved in the attenuation of leptin signalling during late pregnancy in Brandt's voles. Prolactin is a key factor mediating pregnancy-induced leptin resistance in rats (5, 42). Interestingly, exogenous prolactin treatment could induce a marked increase in SOCS3 mRNA in the ARC in ovariectomised rats (43, 44). We therefore speculate that the elevation of hypothalamic SOCS3 mRNA could be involved in the regulation of prolactin-induced leptin resistance.

Leptin-induced activation of POMC neurones is mediated through the JAK/STAT3 pathway, whereas leptin hyerpolarises NPY/AgRP neurones via the involvement of the PI3K/PKB pathway (6, 45). Insufficient melanocortin activation downstream of leptin signalling may be one consequence universal to all forms of leptin resistance (46). In accordance with this concept, hypothalamic POMC mRNA was decreased, whereas hypothalamic mRNA levels of NPY, AgRP and CART showed no significant changes despite high leptin levels in pregnant voles. These findings suggest an apparent paradox because high leptin levels typically stimulate the expression of orexigenic neuropeptides and suppress the expression of anorectic neuropeptides (17). It seems that leptin regulation of these neuropeptides was disrupted in pregnant voles, which lends further support for the notion that leptin signalling may be attenuated or impaired during pregnancy in Brandt's voles. Consistent with this assumption, it has previously been shown that pregnancy-induced leptin resistance was associated with a decrease in leptin-induced activation of STAT3 in the ventromedial hypothalamus, and in the ARC in rats (9). Although the modulations of PI3K/PKB pathway during pregnancy remains unclear, elevated NPY and AgRP mRNA in the presence of high leptin levels in pregnant rats supports the notion that an impaired PI3K/PKB pathway may be one characteristic of pregnancy-induced leptin resistance across species (21, 22).

Progressive decrease in POMC together with the central role in suppressing the feeding response makes this peptide a possible candidate in mediating the hyperphagia during pregnancy in voles. By contrast, the elevation of AgRP in the hypothalamus was shown to be involved in the regulation of pregnancy-induced hyperphagia in rats (22). It would be of interest to identify the discrepancy between the two species in future studies. Prolactin is suggested to be an important component of the increased food intake during pregnancy (5). The injection of prolactin into the third ventricle suppresses POMC mRNA in the ARC (47), making it likely that the orexigenic effect of prolactin could be mediated through the reduction of POMC during pregnancy.

Collectively, the elevation of SOCS3 and the disrupted leptin regulation of neuropeptides in the hypothalamus suggest a state of leptin resistance in pregnant Brandt's voles. However, this awaits further characterisation by exogenous leptin treatment in pregnant Brandt's voles.

Acknowledgements

We thank Professor John R. Speakman, School of Biological Sciences, University of Aberdeen, for his constructive suggestions and for help with improving the English. Quan-Sheng Liu is thanked for his help during the preparation of the manuscript. We are grateful to all the members in Animal Physiological Ecology Group for discussion of the data and their help during the experiment. This study was supported by grants from the National Natural Science Foundation of China (No. 30430140 and 30625009) and National Basic Research Programme of China (2007BC109103) to D.H.W.

Received: 1 February 2008,
revised 26 May 2008,
accepted 28 May 2008

References

1 Johnstone LE, Higuchi T. Food intake and leptin during pregnancy and lactation. *Prog Brain Res* 2001; **133**: 215–227.
2 Speakman JR, Krol E. Limits to sustained energy intake IX: a review of hypotheses. *J Comp Physiol [B]* 2005; **175**: 375–394.
3 Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995; **269**: 543–546.
4 Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 1995; **269**: 540–543.
5 Grattan DR, Ladyman SR, Augustine RA. Hormonal induction of leptin resistance during pregnancy. *Physiol Behav* 2007; **91**: 366–374.
6 Munzberg H, Bjornholm M, Bates SH, Myers MG Jr. Leptin receptor action and mechanisms of leptin resistance. *Cell Mol Life Sci* 2005; **62**: 642–652.
7 Bjorbaek C, Uotani S, da Silva B, Flier JS. Divergent signaling capacities of the long and short isoforms of the leptin receptor. *J Biol Chem* 1997; **272**: 32686–32695.
8 Garcia MD, Casanueva FF, Dieguez C, Senaris RM. Gestational profile of leptin messenger ribonucleic acid (mRNA) content in the placenta and adipose tissue in the rat, and regulation of the mRNA levels of the leptin receptor subtypes in the hypothalamus during pregnancy and lactation. *Biol Reprod* 2000; **62**: 698–703.
9 Ladyman SR, Grattan DR. Suppression of leptin receptor messenger ribonucleic acid and leptin responsiveness in the ventromedial nucleus of the hypothalamus during pregnancy in the rat. *Endocrinology* 2005; **146**: 3868–3874.
10 Szczepankiewicz D, Wojciechowicz T, Kaczmarek P, Nowak KW. Leptin and its receptors in the course of pregnancy in the rat. *Int J Mol Med* 2006; **17**: 95–99.
11 Banks AS, Davis SM, Bates SH, Myers MG Jr. Activation of downstream signals by the long form of the leptin receptor. *J Biol Chem* 2000; **275**: 14563–14572.

12 Bjorbak C, Lavery HJ, Bates SH, Olson RK, Davis SM, Flier JS, Myers MG Jr. SOCS3 mediates feedback inhibition of the leptin receptor via Tyr985. *J Biol Chem* 2000; **275**: 40649–40657.
13 Bjorbaek C, Elmquist JK, Frantz JD, Shoelson SE, Flier JS. Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell* 1998; **1**: 619–625.
14 Tups A, Ellis C, Moar KM, Logie TJ, Adam CL, Mercer JG, Klingenspor M. Photoperiodic regulation of leptin sensitivity in the Siberian hamster, *Phodopus sungorus*, is reflected in arcuate nucleus SOCS-3 (suppressor of cytokine signaling) gene expression. *Endocrinology* 2004; **145**: 1185–1193.
15 Krol E, Tups A, Archer ZA, Ross AW, Moar KM, Bell LM, Duncan JS, Mayer C, Morgan PJ, Mercer JG, Speakman JR. Altered expression of SOCS3 in the hypothalamic arcuate nucleus during seasonal body mass changes in the field vole, *Microtus agrestis*. *J Neuroendocrinol* 2007; **19**: 83–94.
16 Lynn RB, Cao GY, Considine RV, Hyde TM, Caro JF. Autoradiographic localization of leptin binding in the choroid plexus of ob/ob and db/db mice. *Biochem Biophys Res Commun* 1996; **219**: 884–889.
17 Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000; **404**: 661–671.
18 Mercer JG, Speakman JR. Hypothalamic neuropeptide mechanisms for regulating energy balance: from rodent models to human obesity. *Neurosci Biobehav Rev* 2001; **25**: 101–116.
19 Hakansson ML, Brown H, Ghilardi N, Skoda RC, Meister B. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. *J Neurosci* 1998; **18**: 559–572.
20 Tung YC, Piper SJ, Yeung D, O'Rahilly S, Coll AP. A comparative study of the central effects of specific proopiomelancortin (POMC)-derived melanocortin peptides on food intake and body weight in pomc null mice. *Endocrinology* 2006; **147**: 5940–5947.
21 Garcia MC, Lopez M, Gualillo O, Seoane LM, Dieguez C, Senaris RM. Hypothalamic levels of NPY, MCH, and prepro-orexin mRNA during pregnancy and lactation in the rat: role of prolactin. *FASEB J* 2003; **17**: 1392–1400.
22 Rocha M, Bing C, Williams G, Puerta M. Pregnancy-induced hyperphagia is associated with increased gene expression of hypothalamic agouti-related peptide in rats. *Regul Pept* 2003; **114**: 159–165.
23 Ladyman SR. Leptin resistance during pregnancy in the rat. *J Neuroendocrinol* 2008; **20**: 269–277.
24 Li XS, Wang DH. Regulation of body weight and thermogenesis in seasonally acclimatized Brandt's voles (*Microtus brandti*). *Horm Behav* 2005; **48**: 321–328.
25 Zhao ZJ, Wang DH. Short photoperiod influences energy intake and serum leptin level in Brandt's voles (*Microtus brandtii*). *Horm Behav* 2006; **49**: 463–469.
26 Liu H, Wang DH, Wang ZW. Energy requirement during reproduction in female Brandt's voles (*Microtus brandtii*). *J Mammal* 2003; **84**: 1410–1416.
27 Zhang XY, Wang DH. Different physiological roles of serum leptin in the regulation of energy intake and thermogenesis between pregnancy and lactation in primiparous Brandt's voles (*Lasiopodomys brandtii*). *Comp Biochem Physiol C Toxicol Pharmacol* 2008; **Feb 5**: [Epub ahead of print].
28 Ladyman SR, Grattan DR. Region-specific reduction in leptin-induced phosphorylation of signal transducer and activator of transcription-3 (STAT3) in the rat hypothalamus is associated with leptin resistance during pregnancy. *Endocrinology* 2004; **145**: 3704–3711.
29 Bing C, Frankish HM, Pickavance L, Wang Q, Hopkins DF, Stock MJ, Williams G. Hyperphagia in cold-exposed rats is accompanied by decreased plasma leptin but unchanged hypothalamic NPY. *Am J Physiol* 1998; **274**(1 Pt 2): R62–R68.
30 Gavrilova O, Barr V, Marcus-Samuels B, Reitman M. Hyperleptinemia of pregnancy associated with the appearance of a circulating form of the leptin receptor. *J Biol Chem* 1997; **272**: 30546–30551.
31 Chien EK, Hara M, Rouard M, Yano H, Phillippe M, Polonsky KS, Bell GI. Increase in serum leptin and uterine leptin receptor messenger RNA levels during pregnancy in rats. *Biochem Biophys Res Commun* 1997; **237**: 476–480.
32 Ehrhardt RA, Slepetis RM, Bell AW, Boisclair YR. Maternal leptin is elevated during pregnancy in sheep. *Domest Anim Endocrinol* 2001; **21**: 85–96.
33 Henson MC, Castracane VD, O'Neil JS, Gimpel T, Swan KF, Green AE, Shi W. Serum leptin concentrations and expression of leptin transcripts in placental trophoblast with advancing baboon pregnancy. *J Clin Endocrinol Metab* 1999; **84**: 2543–2549.
34 Hardie L, Trayhurn P, Abramovich D, Fowler P. Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. *Clin Endocrinol (Oxf)* 1997; **47**: 101–106.
35 Henson MC, Castracane VD. Leptin in pregnancy. *Biol Reprod* 2000; **63**: 1219–1228.
36 Mistry AM, Romsos DR. Intracerebroventricular leptin administration reduces food intake in pregnant and lactating mice. *Exp Biol Med (Maywood)* 2002; **227**: 616–619.
37 Mounzih K, Qiu J, Ewart-Toland A, Chehab FF. Leptin is not necessary for gestation and parturition but regulates maternal nutrition via a leptin resistance state. *Endocrinology* 1998; **139**: 5259–5262.
38 Howard JK, Cave BJ, Oksanen LJ, Tzameli I, Bjorbaek C, Flier JS. Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. *Nat Med* 2004; **10**: 734–738.
39 Mori H, Hanada R, Hanada T, Aki D, Mashima R, Nishinakamura H, Torisu T, Chien KR, Yasukawa H, Yoshimura A. Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nat Med* 2004; **10**: 739–743.
40 Munzberg H, Flier JS, Bjorbaek C. Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology* 2004; **145**: 4880–4889.
41 Scarpace PJ, Matheny M, Zhang Y, Tumer N, Frase CD, Shek EW, Hong B, Prima V, Zolotukhin S. Central leptin gene delivery evokes persistent leptin signal transduction in young and aged-obese rats but physiological responses become attenuated over time in aged-obese rats. *Neuropharmacology* 2002; **42**: 548–561.
42 Augustine RA, Grattan DR. Induction of central leptin resistance in hyperphagic pseudopregnant rats by chronic prolactin infusion. *Endocrinology* 2008; **149**: 1049–1055.
43 Steyn FJ, Anderson GM, Grattan DR. Hormonal regulation of suppressors of cytokine signalling (SOCS) mRNA in the arcuate nucleus during late pregnancy. *Endocrinology* 2008; **149**: 3206–3214.
44 Anderson GM, Beijer P, Bang AS, Fenwick MA, Bunn SJ, Grattan DR. Suppression of prolactin-induced signal transducer and activator of transcription 5b signaling and induction of suppressors of cytokine signaling messenger ribonucleic acid in the hypothalamic arcuate nucleus of the rat during late pregnancy and lactation. *Endocrinology* 2006; **147**: 4996–5005.
45 Morrison CD, Morton GJ, Niswender KD, Gelling RW, Schwartz MW. Leptin inhibits hypothalamic Npy and Agrp gene expression via a mechanism that requires phosphatidylinositol 3-OH-kinase signaling. *Am J Physiol Endocrinol Metab* 2005; **289**: E1051–E1057.
46 Zhang Y, Scarpace PJ. The role of leptin in leptin resistance and obesity. *Physiol Behav* 2006; **88**: 249–256.
47 Tong Y, Pelletier G. Prolactin regulation of pro-opiomelanocortin gene expression in the arcuate nucleus of the rat hypothalamus. *Neuroendocrinology* 1992; **56**: 561–565.