Octa Journal of Biosciences

journal homepage: www.sciencebeingjournal.com

SPS
Scientific Planet Society

Role of hypothalamic neuropeptides genes expression on body mass regulation under food restriction in *Eothenomys miletus*

Wan-Long Zhu [1], Gaung Yang [2*]

1. Key Laboratory of Ecological Adaptive Evolution and Conservation on Animals-Plants in Southwest Mountain Ecosystem of Yunnan Province Higher Institutes College, School of Life Science of Yunnan Normal University, Kunming; 650500, China;
2. College of Life Sciences of Nanjing Normal University, Nanjing 210023, China

A B S T R A C T

The present study was aimed at examining the role of hypothalamic neuropeptides genes expression on body mass regulation under food restriction in Eothenomys miletus, body mass, food intake, serum leptin levels and hypothalamic neuropeptide Neuropeptide Y (NPY), Agouti Aelated Peptide (AgRP), Pro-opiomelanocortin (POMC), Cocaine and Amphetamine Regulated Transcript (CART) expressions were measured. Food restriction reduced body mass and body fat mass, and increased food intake. Serum leptin levels showed significant differences between control group and food restriction group, and leptin levels showed a positive correlation with body fat mass. Hypothalamic neuropeptide NPY, AgRP, POMC and CART expression showed significant different between two groups. Leptin was negatively correlated with NPY and AgRP, and positively correlated with CART and POMC. All of the results suggested that food restriction can reduce the body mass, body fat mass and leptin levels, increased food intake and hypothalamic neuropeptide expressions. Leptin may play an regulation on body mass and energy metabolism by acting on hypothalamic neuropeptide of NPY/AgRP and CART/CART pathways in E. *miletus*.

INTRODUCTION

Physiological and behavioral plasticity play important roles in adaptation to environmental changes in small mammals (Vezina and Williams 2003). Previous studies showed that morphology and function of organs were related to continuous energy consumption on a long time scale, and were related to aerobic capacity in animals on a short time scale (Speakman and McQueenie 1996). In the natural environment, food resources are essential to the survival of low temperature (Zhao and Cao 2009). Animal in the environment change (including changes in food resources) under the condition of adjusting its body mass, food intake and energy metabolism, but recent studies show that the effects of food restriction on body weight and energy balance for animals is inconsistent (Liang and Zhang 2006), such as food restriction can reduce the body weight of rats (Sucajtys-Szulc et al.. 2008), but did not affect body mass in mice (Hambly and Speakman 2005). Therefore, effects of food resources on energy homeostatic plasticity for animal were not clear in the short time scale.

Leptin can regulate animals' food intake and body mass (Friedman and Halaas 1998). Leptin plays a pivotal role in the regulation of energy intake and energy expenditure in animals (Abelenda et al. 2003), which plays an important role in the maintenance of body mass (Hausman and Barb 2010). In addition, there are studies showed that food restriction can reduce serum leptin levels (Rousseau et al. 2003). The hypothalamic arcuate nucleus (ARC) can regulate food intake under environmental changes (Aguilar et al. 2011). Within the ARC, there are two types of neuropeptides: orexigenic neuropeptides: neuropeptide Y (NPY) and agouti-related protein (AgRP); and anorectic neuropeptides: pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated tran-script (CART); the balance between NPY/AgRP and POMC/CART neuron activities can inhibit food intake and stimulate energy expenditure (Friedman and Halaas 1998). Leptin is mediated by a hierarchy of both anorectic and orexigenic neuropeptidergic neurons in specific sites in the hypothalamus (Arch 2005). Studies have shown that rats fed with high fat food can make NPY and body mass increased significantly (Toshihiro and Akio 2001); long-term hunger increased the content of hypothalamic NPY in rat (Hausman and Barb 2010); lactating rats in the food restricted conditions increased NPY content significantly than that of free feeding group (Abizaid et al., 1997). Long term starvation made the body mass and body fat mass in experimental group decreased significantly, while the content of AgRP increased significantly, and the content of CART and POMC decreased significantly (Henry et al. 2001).

Eothenomys miletus is an inherent species in Hengduan mountain region (Zhu et al. 2010). Previous studies showed that random food deprivation could change body mass, behavior and serum leptin levels in E. *miletus* (Zhu et al. 2014). The aims of this study were to evaluate the role of hypothalamic neuropeptides genes expression on body mass regulation under food restriction in E. *miletus.* We predicted that E. *miletus* may change the expressions hypothalamic neuropeptides to regulate the body mass, and leptin may involve in the regulation of hypothalamic neuropeptide gene expression in E. *miletus* under food restriction.

MATERIALS AND METHODS

Animals and experimental designs

E. *miletus* were obtained from a laboratory colony, which were captured in farmland (26°15´~26°45´N; 99°40´~99°55´E; altitude 2,590m) in Jianchuan County, Yunnan province, 2010. E. *miletus* were maintained at a room temperature of 25±1 °C, under a photoperiod of 12L:12D (with lights on at 08:00), food (standard rabbit pellet chow; produced by Kunming Medical University, Kunming) and water were provided ad libitum. All animal procedures were compliance with the Animal Care and Use Committee of School of Life Science, Yunnan Normal University. This study was approved by the Committee (13-0901-011). Young individuals were excluded in present study. After 1 month of stabilization, 13 male E. miletus were randomly divided into the following two experimental regimes: control group (n=6) that were fed ad libitum during 4 weeks under 25oC, under a photoperiod of 12L:12D (with lights on at 08:00), and food restriction group (n=7) in which each animal was fed food restriction degree 80% under 25°C, under a photoperiod of 12L:2D (with lights on at 08:00) for 4 weeks. On day0 and day28, body mass and food intake were measured, all animals were sacrificed between 0900h and 1100h by decapitation after 28 day, determination of body fat mass, hypothalamic neuropeptide gene expression and serum leptin content. Before the experiment, the body mass between the two groups showed no significant differences (P>0.05). Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus (Zhang and Wang 2007).

Measurement of food intake

Food intake was measured by food equity (Zhao and Cao 2009). Each animal was put in a metabolic cage (20×15×15cm3) with no nest materials, and fed laboratory mice chow pellets. Animals were fed a fixed quantity at a set time(9.5–10.5g, 11:00 am), and the next day body mass was assessed, and residual food collected. Residual food was dried in a vacuum dryer until the mass was invariable.

Measurement of serum leptin levels

Serum leptin levels were determined by radioimmunoassay (RIA) with the 125I Multi-species Kit (Cat. No. XL-85K, Linco Research Inc.). The lowest level of leptin that can be detected by this assay was 1.0 ng/ml when using a 100 µl sample size. And the inter- and intra-assay variability for leptin RIA were <3.6% and 8.7%, respectively.

Measurement of serum leptin levels

Total RNA was isolated from the hypothalamus by using TRIzol Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. To remove any contaminating DNA, RNA samples were treated with DNase I (Promega, USA) at 37°C for 30 min followed by another cycle of TRIzol extraction to eliminate residual DNase I. An equal amount (3 µg) of total RNA was transcribed into first strand cDNA for each sample using the M-MLV First Strand Kit (Invitrogen) according to the manufacturer's instructions.

Primers set for β-actin and four hypothalamic genes(Table 1) were used for real-time q-PCR (Huang et al., 2013). Standard curves were constructed for each gene via serial dilutions of cDNA (1 to 26-fold dilutions). Analysis of standard curves between target genes and β-actin showed that they had similar amplification efficiency, which ensures the validity of the comparative quantity method. Real-time q-PCR was completed using the SYBR Green I qPCR kit (Invitrogen) in the ABI Prism® 7000 Sequence Detection system (Applied Biosystems, Carlsbad CA, USA). Real-time qPCR was carried out in 20 µL reaction agent comprised of 9.5µL RNase-free ddH2O, 9.0 µL Platinum® Quantitative PCR SuperMix-UDG (including Rox), 0.5 µL cDNA templates, 0.5 µL 10 µmoL/L forward primer, and 0.5 µL 10 µmoL/L reserse primer. Each sample was analyzed in triplicate. Thermal cycling conditions were: 50°C for 120 s, 95°C for 120 s, 45 cycles of 95°C for 15 s, and 60°C for 45 s.

Statistical analysis

Data were analyzed using the software package SPSS 15.0. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance using Kolmogorov-Smirnov and Levene tests, respectively. Body mass, food intake, serum leptin levels and hypothalamic neuropeptide gene expressions between control group and food restriction group were analyzed using independent-samples T test. Pearson-correlation analysis was used to detect the relationship between leptin and body fat mass, hypothalamic neuropeptide gene expressions. Results are presented as means ± SEM and $P < 0.05$ was considered to be statistically significant.

RESULTS

Body mass, body fat mass and food intake

Before the experiment, body mass in control group and food restriction group were 45.24±1.65g and 45.82±1.47g, respectively, which showed no significant differences ($t_{1,11}$=-0.26, $P > 0.05$). After 28 days, the body mass had significant differences between the two groups ($t_{1,11}$=3.35, $P < 0.01$, Fig. 1), which reduced 15.86% in food restriction group compared with control group. Body fat mass in 28 days showed significant differences between the two groups ($t_{1,11}$=4.63, $P < 0.01$, Fig. 2) and body fat mass in control group and food restriction group were 6.27±0.49g and 3.43±0.37g, body fat mass in control group reduced 45.29% than that of food restriction group.

Serum leptin levels and hypothalamic neuropeptide gene expressions

Serum leptin levels showed no significant differences between the two groups on 28 day ($t_{1,11}$=3.38, $P<0.05$), and leptin in control group decreased 33.79% than that in food restriction group. Serum leptin levels were positively correlated with body fat mass(r=0.685, $P<0.05$, Fig 3). During the acclimation, NPY, AgRP, POMC and CART expressions had significant difference between the two groups on 28 day (NPY: $t_{1,11}$=-5.176, $P<0.01$; AgRP: $t_{1,11}$=-3.889, $P<0.01$; POMC: $t_{1,11}$=3.453, $P<0.01$, Fig 4). Leptin was negatively correlated with NPY expression (r=-0.568, $P<0.05$ Fig 5a), with AgRP expression (r=-0.623, $P<0.05$, Fig 5b) and POMC expression (r=0.678, $P<0.05$, Fig 6a) and with CART expression (r=0.555, $P<0.05$, Fig 6b).

Table 1: Gene-Specific Primers Used for Real-Time qPCR

Primer	Oligonuncleotide sequence (5' to 3')	Product size (bp)
NPY (forward)	TGGACTGACCCTCGCTCTAT	162
NPY(reverse)	GTGTCTCAGGGCTGGATCTC	
AgRP(forward)	AGAGTTCTCAGGTCTAAGTCT	187
AgRP(reverse)	CTTGAAGAAGCGGCAGTAGCACGT	
POMC(forward)	CCTGTGAAGGTGTACCCAATGTC	240
POMC(reverse)	CACGTTCTTGATGATGGCGTTC	
CART(forward)	AGAAGAAGTACGGCCAAGTCC	55
CART(reverse)	CACACAGCTTCCCGATCC	
β-actin(forward)	GAGAGGGAAATCGTGCGTGAC	170
β-actin (reverse)	CATCTGCTGGAAGGTGGACA	
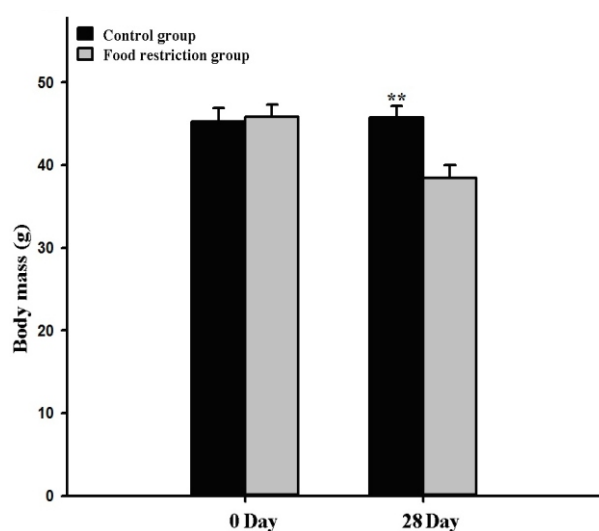
Figure 1. Effect of food restriction on body mass in *Eothenomys miletus*.
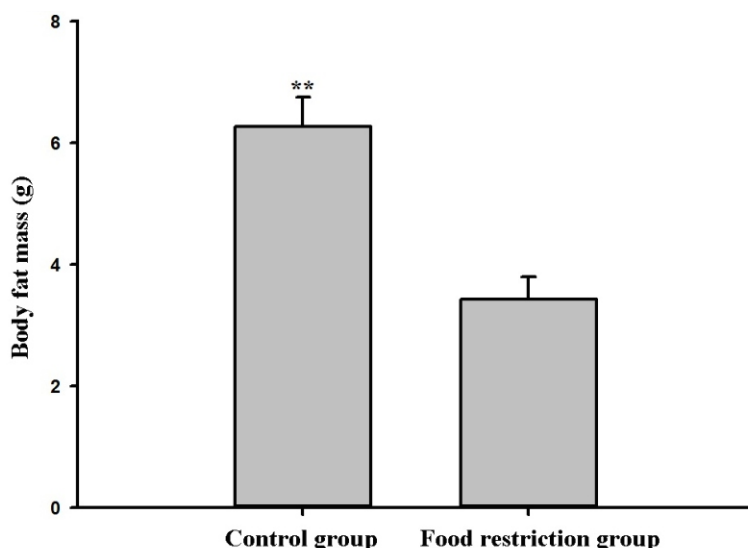** significant difference (P<0.01) between the two groups

Figure 2. Effect of food restriction on body fat mass in *Eothenomys miletus*.
** significant difference (P<0.01) between the two groups

DISCUSSION

Under different conditions of food quantity and quality, there will be a significant change in body weight in small mammals (Zhang and Wang 2008). Studies showed food restriction affect body mass in animals: if food restriction to a lesser extent, the animal can maintain energy homeostasis and body weight homeostasis. This is mainly by increasing the gastrointestinal absorption capacity, adjust heat production and activity; on the contrary, if food restriction greatly, animals will not be able to maintain energy homeostasis, which leads to weight decreased (Hausman and Barb 2010).

In the present study, E. *miletus* decreased body mass significantly in food restriction group, which was consistent with the results of Kunming mice (Zhao et al. 2009). Food restriction had significant effect on body fat mass of E. miletus, indicating that E. *miletus* need fat mobilization to make up the heat produced for the lack of food.

Leptin plays an important role in the regulation of body mass in small mammals (Abelenda et al. 2003). Leptin content can reflect the content of adipose tissue (Schneider et al. 2000). Current research indicates that there had a positive relationship between serum leptin level and body fat mass (Zhao 2011). In our study, it showed a positive
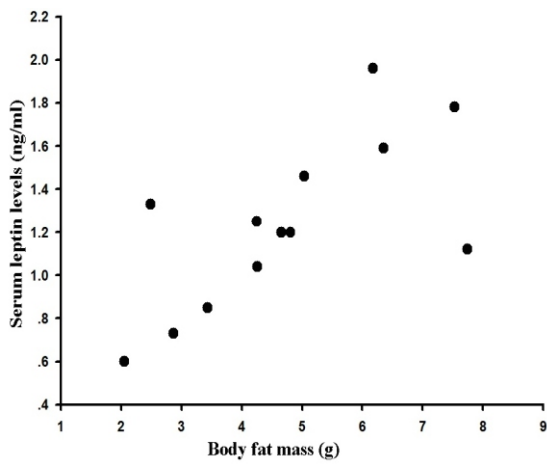
Figure 3: Correlation between serum leptin levels and body fat mass in *Eothenomys miletus*
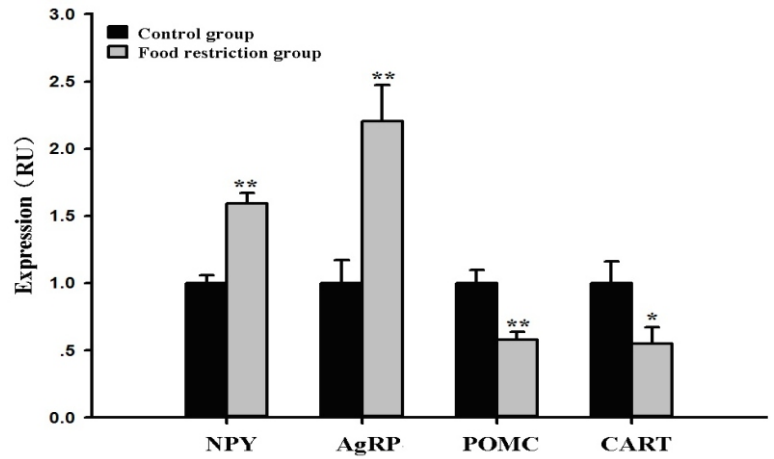

Figure 4: Effect of food restriction on NPY , AgRP , POMC and CART in *Eothenomys miletus*
* significant difference (P<0.05), ** significant difference (P<0.01) between the two groups
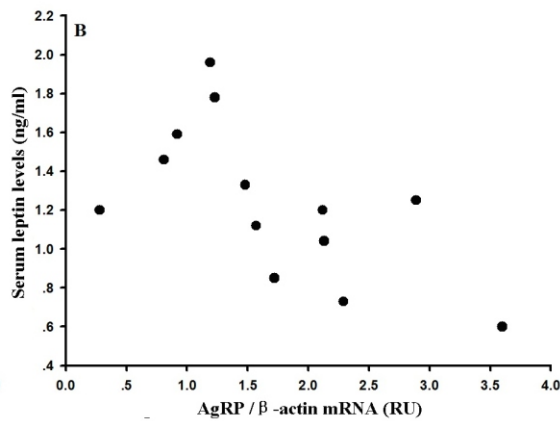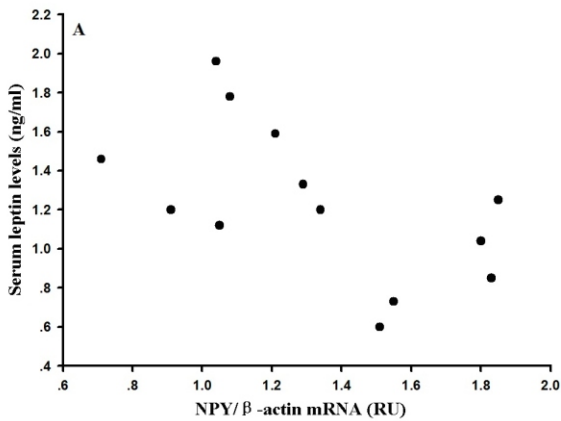

Figure 5: Correlation of NPY (A) and AgRP (B) with serum leptin level in *Eothenomys miletus* under food.
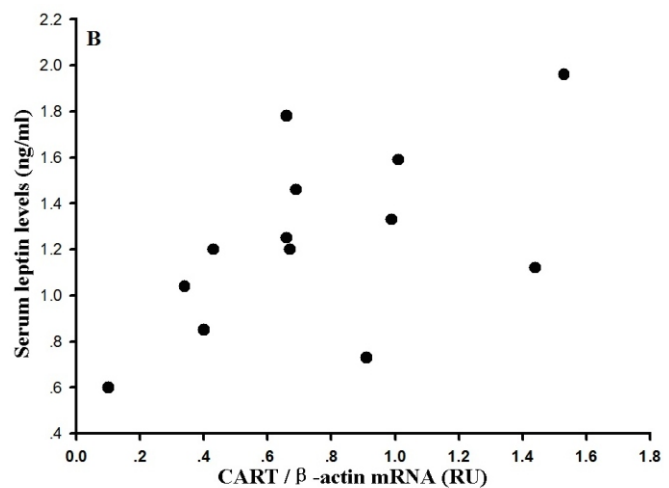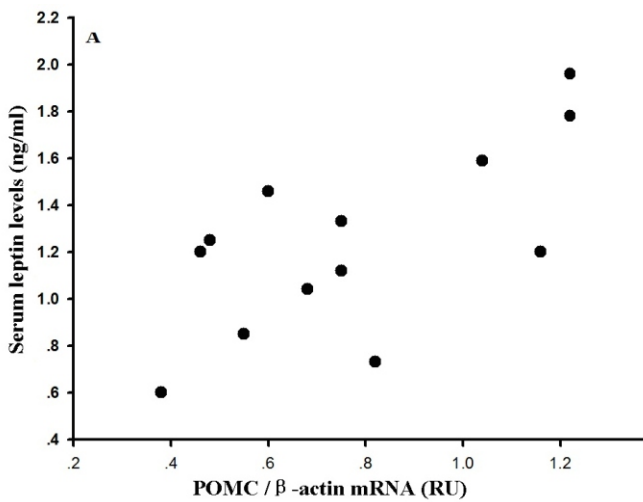

Figure 6 : Correlation of POMC (A) and CART (B) with serum leptin level in *Eothenomys miletus* in under food restriction.

correlation between leptin and fat content, supporting the leptin can be used as a lipid signaling molecule (Zhao and Wang 2006). Previous studies showed that the NPY content of hypothalamus in rats increased in the long term food restriction (Hausman and Barb 2010), and the content of AgRP in rats and Siberia hamsters increased under food restriction (Harrold et al. 1999; Mercer and Tups 2003). However, studies have shown that high fat foods can increase body mass, leptin content significantly in rat, but had no effect on content of POMC (Marco et al. 2013), and in rats chronically fed with high fat diet, POMC content of their offspring increased significantly compared with the control group (Lukaszewski et al. 2013). In addition, fasting can also make the expression of CART in rats decreased (Tian et al. 2004). Hunger reduced the expression of CART and increased appetite in mice (Yoo et al. 2011). In the present study, it showed that food restriction had significant effect on hypothalamic neuropeptide gene expression, suggesting that E. *miletus* in food restricted environment through increased NPY and AgRP

expression to increase appetite, reduce heat production, in order to maintain the survival. Food restriction decreased the expression of POMC and CART, which were help to increase food intake. Through analysis of correlation between leptin levels and hypothalmic neuropeptide gene was also confirmed that leptin involved in and NPY/AgRP POMC/CART pathways regulation under food restricted conditions, thereby regulating body mass and energy metabolism in E. *miletus*.

CONCLUSION

In conclusion, E. *miletus* reduced body mass, body fat mass and leptin levels, increased NPY and AgRP expression significantly, and reduced POMC and CART expression significantly under food restriction. Leptin was positively correlated with body fat mass, POMC and CART expression, and negatively correlated with the expression of NPY and AgRP. These results suggest that food restriction affect body

mass and energy metabolism in E. *miletus*, and leptin regulate energy homeostasis by regulating hypothalamic neuropeptide gene expression in E. *miletus*.

REFERENCES

1. Abelenda, M., Ledesma, A., Rial, E (2003). Leptin administration to cold acclimated rats reduces both food intake and brown adipose tissue thermogenesis. *J. Therm. Biol.*, 28: 525-530.
2. Abizaid, A., Walker, C.D., Woodside, B (1997). Changes in neuropeptide Y immunoreactivity in the arcuate nucleus during and after food restriction in lactating rats. *Brain Research*, 761: 306-312.
3. Aguilar, A.J., Conde-Sieira, M., López-Patiño, M.A., Míguez, J.M., Soengas, J.L (2011). In vitro leptin treatment of rainbow trout hypothalamus and hindbrain affects glucosensing and gene expression of neuropeptides involved in food intake regulation. *Peptides*, 32: 232-240.
4. Arch, J.R (2005); Central regulation of energy balance: inputs, outputs and leptin resistance. *P. Nutr. Soc.*, 64: 39-46.
5. Friedman, J.M., Halaas, J.L (1998). Leptin and the regulation of body weight in mammals. *Nature*, 395: 763-770.
6. Hambly, C., Speakman, J.R (2005). Contribution of different mechanisms to compensation for energy restriction in the mouse. *Obesity Research*, 13: 1548-557.
7. Harrold, J.A., Williams, G., Widdowson, P.S (1999). Changes in hypothalamic agouti-related protein (AGRP), but not α-MSH or pro-opiomelanocortin concentrations in dietary-obese and food-restricted rats. *Biochemical and Biophysical Research Communications*, 258: 574-577.
8. Hausman, G.J., Barb, C.R (2010). Adipose tissue and the reproductive axis: biological aspects. In: Levy-Marchal C, Penicaud L (eds) Adipose tissue development: from animal models to clinical conditions. Endocrine development, vol 19. Karger, Basel, 31-44.
9. Henry, B.A., Rao, A., Ikenasio, B.A (2001). Differential expression of cocaine- and amphetamine-regulated transcript and agouti related-protein in chronically food-restricted sheep. *Brain Research*, 918: 40-50.
10. Huang, C.M., Zhu, W.L., Yu, T.T., Yang, S.C., Wang, Z.K (2013). Amplification and sequence analysis of partial cDNA sequence of NPY, AgRP, POMC and CART genes in Eothenomys miletus. *Acta Theriol. Sin.*, 33: 186-192.
11. Liang, H., Zhang, Z.B (2006). Food restriction affects reproduction and survival of F1 and F2 offspring of Rat-like hamster (Cricetulus triton). *Physiological Behaviour*, 87: 607-613.
12. Lukaszewski, M.A., Butruille, L., Moitrot, E (2013). The hypothalamic POMC mRNA expression is upregulated in prenatally undernourished male rat offspring under high-fat diet. *Peptides*, 43: 146-154.
13. Marco, A., Kisliouk, T., Weller, A (2013). High fat diet induces hypermethylation of the hypothalamic Pomc promoter and obesity in post-weaning rats. Psychoneuroendocrinology, 38: 2844-2853.
14. Mercer, J.G., Tups, A (2003). Neuropeptides and anticipatory changes in behavior and physiology: seasonal body weight regulation in the Siberian hamster. *European Journal of Pharmacology*, 480: 43-50.
15. Rousseau, K., Actha, Z., Loudon, A.S.I (2003). Leptin and seasonal mammals. *J. Neuroendocrinology*, 15: 409-414.
16. Schneider, J.E., Zhou, D., Blum, R.M (2000). Leptin and metabolic control of reproduction. *Horm. Behav.*, 37: 306-326.
17. Speakman, J.R., McQueenie, J (1996). Limits to sustained metabolic rate: the link between food intake, basal metabolic rate and morphology in reproducing mice, Mus musculus. *Physiological Zoology*, 69: 746-769.
18. Sucajtys-Szulc, E., Goyke, E., Korczynska, J (2008). Chronic food restriction differentially affects NPY mRNA level in neurons of the hypothalamus and in neurons that innervate liver. *Neuroscience Letters*, 433: 174-177.
19. Tian, D.R., Li, X.D., Shi, Y.S (2004). Changes of hypothalamic alpha-MSH and CART expression in diet-induced obse rats. *Peptides*, 25: 2147-2153.
20. Toshihiro, K., Akio, I (2001);. Modest overexpression of neuropeptide Y in the brain leads to obesity after high-suerose feeding. *Diabetes*, 50: 1206-1210.
21. Vezina, F., Williams, T.D (2003). Plasticity in body composition in breeding birds: what drives the metabolic costs of egg production? *Physiological and Biochemical Zoology*, 76: 716-730.
22. Yoo, S.B., Ryu, V., Park, E.Y (2011); The arcuate NPY, POMC, and CART expressions responding to food deprivation are exaggerated in young female rats that experienced neonatal maternal separation. *Neuropeptides*, 45: 343-349.
23. Zhang, X.Y., Wang, D.H (2007). Thermogenesis, food intake and serum leptin in cold-exposed lactating Brandt's voles Lasiopodomys brandtii. *J. Exp. Biol.*, 210: 512-521.
24. Zhang, L.N., Wang, D.H (2008). Effects of food restriction and refeeding on energy balance regulation in Mongolian gerbils (Meriones unguiculatus). BFDG Abstracts/Appetite, 51: 751-764.
25. Zhao, Z.J., Wang, D.H (2006). Effects of photoperiod on energy budgets and thermogenesis in Mongolian gerbils (Meriones unguiculatus). *J. Therm. Biol.*, 31: 323-331.
26. Zhao, Z.J., Cao, J (2009). Effect of fur removal on the thermal conductance and energy budget in lactating Swiss mice. *J. Exp. Biol.*, 212: 2541-2549.
27. Zhao, Z.J., Cao, J., Wang, G.Y (2009). Effect of random food deprivation and re-feeding on energy metabolism and behavior in mice. *Acta Theriologica Sinica*, 29: 277-285.
28. Zhao, Z.J (2011). Serum leptin, energy budget and thermogenesis in striped hamsters exposed to consecutive decrease in ambient temperatures. *Physiol. Biochem. Zool.*, 84: 560-572.
29. Zhu, W.L., Cai, J.H., Lian, X., Wang, Z.K (2010); Adaptive character of metabolism in Eothenomys miletus in Hengduan Mountains region during cold acclimation. *Journal of Thermal Biology*, 35: 417-421.
30. Zhu, W.L., Mu, Y., Zhang, H., Wang, Z.K (2014); Effects of random food deprivation on body mass, behavior and serum leptin levels in Eothenomys miletus (Mammalia: Rodentia: Cricetidae). *Italian Journal of Zoology*, 81: 227-234.