ORIGINAL PAPER

Seasonal regulations of energetics, serum concentrations of leptin, and uncoupling protein 1 content of brown adipose tissue in root voles (*Microtus oeconomus*) from the Qinghai-Tibetan plateau

Jian-Mei Wang · Yan-Ming Zhang · De-Hua Wang

Received: 28 December 2005 / Revised: 22 February 2006 / Accepted: 18 April 2006 / Published online: 20 June 2006 © Springer-Verlag 2006

Abstract Survival of small mammals in winter requires proper adjustments in physiology, behavior and morphology. The present study was designed to examine the changes in serum leptin concentration and the molecular basis of thermogenesis in seasonally acclimatized root voles (Microtus oeconomus) from the Qinghai-Tibetan plateau. In January root voles had lower body mass and body fat mass coupled with higher nonshivering thermogenesis (NST) capacity. Consistently, cytochrome c oxidase activity and mitochondrial uncoupling protein-1 (UCP1) protein contents in brown adipose tissues were higher in January as compared to that in July. Circulating level of serum leptin was significantly lower in winter and higher in July. Correlation analysis showed that serum leptin levels were positively related with body mass and body fat mass while negatively correlated with UCP1 protein contents. Together, these data provided further evidence for our previous findings that root voles from the Qinghai-Tibetan plateau mainly depend on higher

Communicated by I.D. Hume

J.-M. Wang · Y.-M. Zhang Northwest Plateau Institute of Biology, Chinese Academy of Sciences, Xining 810001 Qinghai, China

D.-H. Wang $(\boxtimes) \cdot J.-M.$ Wang

State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, 25 Beisihuan Xilu, Haidian Beijing 100080, China e-mail: wangdh@ioz.ac.cn

J.-M. Wang

Graduate School of the Chinese Academy of Sciences, Beijing 100049, China

NST coupled with lower body mass to enhance winter survival. Further, fat deposition was significantly mobilized in cold winter and leptin was potentially involved in the regulation of body mass and thermogenesis in root voles. Serum leptin might act as a starvation signal in winter and satiety signal in summer.

Keywords Basal metabolic rate (BMR) · Leptin · Nonshivering thermogenesis (NST) · Root vole (*Microtus oeconomus*) · Uncoupling protein 1 (UCP1)

Introduction

Winter is an energetically stressful period for small mammals in temperate zones because cost of thermoregulation was increased while food quality and availability were reduced (Scantlebury et al. 2005). To cope with the harsh conditions, many winter-active rodent species such as Brandt's voles (*Microtus brandti*) (Li and Wang 2005a), Siberian hamsters (*Phodopus sungorus*) (Klingenspor et al. 2000), the bushy-tailed gerbils (*Sekeetamys calurus*) (Haim 1996), wild bank voles (*Clethrionomys glareolus*) (Klause et al.1988), and white-footed mice (*Peromyscus leucopus*) (Lynch 1973) enhanced their thermogenic capacities coupled with decline in body mass.

Nonshivering thermogenesis (NST) is a dominant pathway for thermoregulatory heat production to defend against the cold winter (Jansky 1973) and brown adipose tissue (BAT), though the activity of uncoupling protein 1 (UCP1), is the main site for NST. UCP1, a 32-kDa carrier protein unique in the mitochondrial inner membrane of brown adipocytes, is essential for adaptive thermogenesis in small mammals (Nicholls and Locke 1984; Cannon and Nedergaard 2004; Krauss et al. 2005). It has been found that some small mammals showed enhanced NST associated with increased UCP1 mRNA level or UCP1 protein contents in winter conditions (Li et al. 2001; Praun et al. 2001; Li and Wang 2005a).

Leptin, a 167-amino acid product of the ob gene and secreted mainly by the adipocytes of mammals (Zhang et al. 1994; Dijk 2001), is an important regulator of body mass via its control on food intake and energy expenditure by acting on neuropeptide circuits in the hypothalamus (Friedman and Halaas 1998; Paracchini et al. 2005). Some small mammal species such as Siberian hamsters (Mercer et al. 2000) and Brandt's voles (Li and Wang 2005a; Zhao and Wang 2005) decreased leptin levels significantly in winter-like conditions, whereas collared lemmings (Dicrostonyx torquatus) (Johnson et al. 2004) and Syrian hamsters (Mesocricetus auratus) (Buckley and Schneider 2003) increased leptin levels under winter conditions, suggesting species-specific responses in leptin level to seasonal acclimatization. Although seasonal regulations in body mass and thermogenesis were well documented in laboratory animals or in several wild species under laboratory conditions, only few data on seasonal adjustments in small wild mammals are available. Furthermore, only a few studies integrated the studies on thermogenesis and body mass regulation for wild rodents.

Root voles (Microtus oeconomus) are widely distributed species, occurring from northwest Europe, eastward through north and central Asia to Alaska and northwest Canada (Brunhoff et al. 2003). They are herbivorous and winter-active species and live in the Potentilla fruticosa shrub of alpine meadows on the Qinghai-Tibetan plateau, China. Qinghai-Tibetan plateau is unique, with its high altitude coupled with extreme harsh environmental conditions, such as cold and hypoxia as well as the low productivity. It has been found that root voles showed seasonal changes in basal metabolic rate (BMR), NST, BAT thermogenic properties and body mass (Jia and Sun 1986; Wang and Wang 1989, 1996, 2000; Wang et al. 1991, 1996). However, there is still no molecular evidence to interpret the seasonal changes in thermogenesis at organismal level in this species. Further, it remains unknown whether there are seasonal changes in leptin levels that might participate in the physiological regulation. Here we carried out an integrative study from molecular to organismal levels and combined the changes in thermogenesis and serum leptin concentrations in this species. We hypothesized that leptin was involved in the regulation of body mass and changes of UCP1 protein content in BAT were consistent with the variations in NST in root voles.

Materials and methods

Animals

All experimental procedures were licensed by the Animal Care and Committee of the Northwest Plateau Institute of Biology, Chinese Academy of Sciences. Root voles (five males and five females) were captured during the middle ten days in April, July and October 2004 and January 2005, at alpine meadow around the Haibei Alpine Meadow Ecosystem Research Station, the Chinese Academy of Sciences (37°29'-37°45' N, 101°12'-101°33'E, 3,200-3,500 m in altitude) at Menyuan County, Qinghai Province. All pregnant, lactating or young individuals (body mass below 16 g) were excluded. The annual mean temperature is -0.4 to -2.5°C (Li et al. 2004). Ambient temperature fluctuates dramatically either everyday or across seasons. It was reported that the highest ambient temperature reached 26.8° C, and the lowest fell to -37.1° C (Li et al. 2004). The plant growing period lasts only about 134 days. Climatic characteristics on the alpine meadow were shown in Fig. 1.

After being captured, root voles were transported to the Northwest Plateau Institute of Biology, the Chinese Academy of Sciences in Xining, Qinghai, China (2,275 m in altitude). Animals were kept individually in plastic cages ($290 \times 180 \times 160$ mm) with sawdust as bedding. Before being killed, the animals were kept in a room with natural temperature and natural photoperiod and fed on natural grazing and carrot ad libitum. After all the oxygen consumption measurements were finished within 3 days, animals were killed by puncturing the posterior vena cava between 900 and 1100 hours, and blood and tissue samples were prepared for further physiological analysis.

Metabolic trials

Metabolic measurements were measured as oxygen consumption and conducted with an established closed-circuit respirometer 1 day after the animals were transported into laboratory in Xining (Wang and Wang 1996). Animals were starved for 3 h and then kept in the metabolic chamber for 1 h stabilization prior to the basal metabolic rate (BMR) measurement. BMR was measured at 30°C, which was within the thermoneutral zone for this species (28–32.5°C; Wang and Wang 2000). NST was induced



by subcutaneous injections of norepinephrine bitartrate (NE) (Shanghai Harvest Pharmaceutical Co.Ltd) and measured at $25 \pm 1^{\circ}$ C. NE dosage was calculated according to the equation: (mg/ kg) = 6.6 Mb $^{-0.458}$ (g) (Heldmaier 1971), which was reported to be able to induce the maximum NST in this species (Wang and Wang 1996; Wang et al. 1999). Zeng et al. (1981) reported that the most activities for root voles occured at 0600 and 2400 hours under laboratory conditions with sufficient food supply. Hence, in this study, all metabolic measurements were performed between 1000 and 1700 hours to minimize the effect of circadian rhythms. Briefly, the metabolic chamber size was 3.61. Water bath was used to control the temperature ($\pm 0.5^{\circ}$ C) and KOH and silica gel were used to absorb carbon dioxide and water in the metabolic chamber. Both BMR and NST lasted for 60 minutes with an interval at every 5 minutes to record the reading. The two stable consecutive lowest readings were used to calculate BMR and the two stable consecutive highest readings were recorded for NST calculation (Li and Wang 2005a; Zhao and Wang 2005). All oxygen consumption data were corrected to standard temperature and pressure (STP). Animals' body temperature and body mass were measured before and after each test.

Measurements of cytochrome c oxidase (COX) activity and mitochondrial protein content

After the animals were sacrificed between 0900 and 1100 hours, the interscapular BAT were removed and weighed immediately for mitochondrial protein preparation as described previously (Wang et al. 1999; Li et al. 2001). Mitochondrial protein concentrations were determined by the Folin phenol method (Lowry et al. 1951) with bovine serum albumin as the standards. The COX activity was measured with the polarographic method using oxygen electrode units (Hansatech Instruments Ltd., England) (Li and Wang 2005a; Zhao and Wang 2005).

Measurement of UCP1 protein content and serum leptin level

Five milligram BAT mitochondrial protein $(4 \mu g/\mu l)$ was diluted in 5 µl s.ple buffer (0.125 M Tris-HCl, pH6.8, 4% SDS, 0.2 M DTT, 20% Glycerol, and 0.2% bromophenol blue) and run on a SDS-polyacryladmide gel (3% stacking gel and 12.5% running gel) for 2 h together with a prestained protein marker. UCP1 was detected using a polyclonal rabbit anti-hamster UCP1 (1:5,000, supplied by Dr. M. Klingenspor, Department of Biology, Philipps-University Marburg, Germany) as a primary antibody and peroxidase-conjugated goat anti-rabbit (1:5,000, Jackson Immuno. Inc., USA) as the second antibody (Klingenspor et al. 1996). Enhanced chemoluminescence (ECL, Amersham) were used to detect specific binding sites. UCP1 protein contents was quantified with Scion Image Software (Scion Corporation) and expressed in relative units (RU) (Li and Wang 2005a; Zhao and Wang 2005).

Serum was separated from blood samples and stored at -75° C. Serum leptin levels were measured by radioimmunoassay (RIA) using the Linco ¹²⁵I multispecies Kit (Linco, St. Louis, MO, USA). The lower and upper detecting limit for leptin were1.0 and 50 ng/ml. Inter- and intra-assay variability for leptin RIA were < 3.6 and 8.7%, respectively.

Body fat analysis

The eviscerated carcass without BAT and the entire gastrointestinal tract was oven-dried at 60°C for 3 days to get constant mass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus (Li and Wang 2005a).

Statistical analysis

Data were analyzed with the SPSS software package (Windows version 13.0). Distributions of all variables

were tested for normality by the Kolmogorov-Smirnov test. Sex differences were analyzed by two-way (season by gender) analysis of variance (two-way ANOVA for BMR,NST, Mitochondrial protein content and UCP1 protein content or two-way ANCOVA for BAT mass and body fat mass as well as serum leptin level). If no gender differences were detected, seasonal variations in parameters were analyzed by one-way analysis of variance (ANOVA) except that seasonal differences in BAT mass and body fat mass as well as serum leptin level were tested by analysis of covariance (ANCO-VA) with body mass as the covariate. Group differences were further evaluated by least-significant difference (LSD) post hoc test. Pearson correlation was performed to detect possible correlations between serum leptin and body mass, body fat mass, and UCP1 content. All values were expressed as mean \pm SE and statistical significance was determined at P < 0.05.

Results

No significant sex differences were found on any of the measurements. Therefore, data on males and females were combined.

Body mass and body fat mass

The root voles showed significant variations in body mass among seasons ($F_{(3,36)} = 18.285$, P < 0.001,

Fig. 2 Seasonal changes of body mass (a), body fat mass (b) and body fat percent (c) in root voles. Body fat mass was positively correlated with body mass (d). P < 0.05 was considered to be statistical significant

Fig 2a). Body mass was highest in October and lowest in January. Body mass in January was 31.5 and 17.3% lower as compared to that in October and July respectively. Significant seasonal variations were found in body fat mass ($F_{(3,35)} = 3.174$, P < 0.05, Fig. 2b). In July and October, body fat mass was significantly higher than that in January, whereas no differences were found between April and January groups. Body fat (in percentage of body mass) in October was significantly higher than that in January and April, and no differences were found between July and other time points ($F_{(3,36)} = 2.925$, P < 0.05, Fig. 2c). Further, body fat mass was positively correlated with overall body mass ($R^2 = 0.830$, P < 0.001, Fig 2d).

BMR and NST

No significant variations in body temperature were found across seasons $(F_{(3,36)} = 0.162, P > 0.05, Ta$ ble 1). BMR and NST were shown as oxygen consumption per individual (mlO₂/h; Fig. 3a, for original data of oxygen consumption, Fig 3b shows the results of oxygen consumption adjusted with body mass as covariate). Significant seasonal changes were found in BMR per individual ($F_{(3,35)} = 5.916, P > 0.001$), which was higher in October and July than that in January and April. Further, NST showed marked differences across The NST individual seasons. per $(F_{(3,35)} = 14.688, P < 0.001)$ was significantly higher in January than that in July.



Table 1 Seasonal variations in body temperature, BAT mass, mitochondrial protein content (MP) and COX activity in root voles

Group	April	July	October	January	Р
Sample size	10	10	10	10	
Body temperature (°C)	37.6 ± 0.5	37.8 ± 0.4	37.7 ± 0.5	37.2 ± 0.7	ns
BAT mass (g)	$0.067 \pm 0.007^{\rm b}$	0.103 ± 0.012^{a}	$0.067 \pm 0.007^{\rm b}$	$0.065 \pm 0.007^{\rm b}$	0.01
BAT MP content					
mg/g BAT	$10.203 \pm 0.824^{\circ}$	6.715 ± 0.582^{d}	14.487 ± 0.642^{b}	$18.955 \pm 0.776^{\rm a}$	0.001
mg in whole BAT	0.653 ± 0.066^{b}	0.724 ± 0.124^{b}	0.882 ± 0.105^{ab}	1.228 ± 0.146^{a}	0.01
BAT COX activity					
nmolO ₂ /min/mg MP	152.964 ± 25.572 ^{ab}	134.718 ± 6.542^{b}	151.209 ± 5.510^{ab}	195.383 ± 7.941^{a}	0.05
nmol O ₂ /min/g BAT	$1388.726 \pm 81.767^{\circ}$	874.461 ± 39.653^{d}	2186.437 ± 114.831^{b}	3701.226 ± 199.221^{a}	0.001
nmolO ₂ /min in whole BAT	$96.015 \pm 12.008^{\rm bc}$	$67.472 \pm 7.488^{\circ}$	$131.093 \pm 13.747^{\rm b}$	242.022 ± 31.258^{a}	0.001

Means with the same superscripts within rows are not significantly different (P > 0.05)

Fig. 3 Seasonal changes of basal metabolic rate (*BMR*; **a**, **b**), nonshivering thermogenesi (*NST*, **a**, **b**), uncoupling protein 1 (*UCP1*, **c**) content and correlation between body *UCP1* protein content with mitochondrial protein content in BAT (**d**) in root voles. P < 0.05 was considered to be statistically significant





BAT mass ($F_{(3,35)} = 4.874$, P < 0.01) showed significant variations across seasons. The highest occurred in July and no significant differences were found among other groups (Table 1). No correlation was found between BAT mass and body mass ($R^2 = 0.014$; P > 0.05).

Seasonal variations in mitochondrial protein contents were found either in mg per g BAT ($F_{(3,36)} = 55.306$, P < 0.001) or in mg per individual ($F_{(3,36)} = 5.041$, P < 0.01,). The mitochondrial protein contents were highest in January as compared to July (Table 1). Cytochrome c oxidase (COX) activity showed significant seasonal variations (Table 1). The COX activities per mg mitochondrial protein ($F_{(3,36)} = 3.391$, P < 0.05), per g BAT ($F_{(3,36)} = 99.536$, P < 0.001) and total COX activities in whole BAT ($F_{(3,36)} = 17.122$, P < 0.001) in January were significantly higher than that in July.

There were significant seasonal differences in UCP1 protein contents in BAT ($F_{(3,36)} = 29.623$, P < 0.001; Fig. 3c), which were higher in January and October than that in July and April. UCP1 protein contents were positively correlated with mitochondrial protein contents in BAT (mg/g BAT; $R^2 = 0.435$; P < 0.001),

and not correlated with body mass ($R^2 = 0.022$; P > 0.05) or body fat mass ($R^2 = 0.048$; P > 0.05).

Serum leptin

Significant seasonal variations were found in serum leptin levels (the sample size in serum leptin measurement was five; three females and two males for July and October, four females and one male for April, two females and three males for January) ($F_{(3,15)} = 3.298$, P < 0.05, Fig. 4a). In July and October, serum leptin levels were significantly higher than that in January, whereas no differences were found between April and January. Correlation analysis showed that serum leptin levels were positively correlated with overall body mass ($R^2 = 0.437$, P < 0.01; Fig. 4b), body fat mass ($R^2 = 0.601$, P < 0.001; Fig. 4c), and negatively correlated with UCP1 contents ($R^2 = 0.354$, P < 0.01; Fig. 4d).

Discussion

Root voles living in the Qinghai-Tibetan plateau faced extremely harsh environmental stress (cold and hypoxia) and had evolved several strategies to cope with the environment. Our present study found that root voles showed seasonal changes in UCP1 protein contents in BAT, which was consistent with seasonal variation in NST. Serum leptin levels were lower in winter

J Comp Physiol B (2006) 176:663-671

and higher in summer and body mass and body fat mass were lowest in winter. The seasonal variations in serum leptin level coupled with its correlation with body fat mass and UCP1 protein content indicated that leptin was potentially involved in the regulation of body mass and energy expenditure in this species.

Seasonal variations inUCP1 protein contents and thermogenic capacities

Small mammals largely depend on NST to cope with cold stress (Jansky 1973; Heldmaier et al. 1982). Winter-induced enhancement in NST has been reported in some small mammal species under natural, semi-natural and laboratory conditions (Feist and Feist 1986; Bozinovic et al. 2004; Jefimow et al. 2004a, b). Our present study found that root voles showed significantly higher NST in January (winter) than that in July (summer), which was consistent with the previous findings (Wang and Wang 1996).

NST capacity mainly depends on the concentration of UCP1 in BAT (Barbara and Nedergaard 2004). UCP1 had a strongly regulated uncoupling activity (Ricquier and Bouilloud 2000). It has been found that some small mammals showed enhanced NST associated with increased UCP1 mRNA level or UCP1 protein contents in winter conditions, such as common spiny mice (*Acomys cahirinus*) (Kronfeld-Schor et al. 2000), Siberian hamster (Praun et al. 2001), and Mongolian gerbil (Li et al. 2001). In the present study,

Fig. 4 Seasonal variations in serum leptin levels (\mathbf{a} , n = 5) and its correlations with body mass (\mathbf{b}), body fat mass (\mathbf{c}) and UCP1 content (\mathbf{d}). P < 0.05 was considered to be statistically significant



UCP1 protein contents in BAT were lower in July and kept higher from October to January, which was consistent with the seasonal changes in NST. Correlation analysis showed that UCP1 protein contents were positively correlated with mitochondrial protein contents in BAT whereas they did not correlate with body mass or body fat mass, indicating that seasonal changes in UCP1 protein contents were consistent with seasonal variations in thermogenic parameters in BAT, and were not induced by the seasonal differences in body mass or fat mass. This is the first report that provides the molecular evidence for the enhancement in thermogenic capacity in winter voles. Our present study showed that UCP1 protein content in BAT was increased by 194% in January (winter) as compared to that in July (summer), whereas in Brandt's voles UCP1 protein content was elevated by 43% in winter (October to February) than that in summer (June to August) (Li 2005; Li and Wang 2005a), and UCP1 contents of Mongolian gerbils under semi-natural conditions increased 77% in winter (November to February) than that in summer (June to August) (Li 2005) and 65% higher in winter than in summer for Mongolian gerbils in the field (Zhang, 2005), which might indicate that the extreme harsh environment wherein the root voles live, required more cost for thermoregulation.

It is interesting, that BMR in root voles showed no winter-associated enhancement, which was different from that of other rodent species such as prairie voles (Microtus ochrogaster) (Wunder et al. 1977), red-backed voles (Clethrionomys rutilus) (Feist and Feist 1986) and Mongolian gerbils (Li et al. 2001; Li and Wang 2005b) as well as Brandt's voles (Li and Wang 2005a; Zhao and Wang 2005), which increased BMR in winter-like conditions. Similar strategy was also found in plateau pikas (Ochotona curzoniae) and Gansu pikas (Ochotona cansus), the sympatric lagomorpha species native to the alpine meadows on the Qinghai-Tibetan plateau (Wang et al. 1979; Wang et al. 1991; Wang and Wang 1996; Wang and Wang 2000), indicating that this kind of strategy may be common among small alpine mammals to cope with the harsh environment in the Qinghai-Tibetan plateau meadow. It has been reported that small mammals living in the high altitude and cold environments showed high basal metabolism levels. BMR in root voles was 205-220% of the predicted values based on their body mass (Jia and Sun 1986). Similarly other sympatric alpine small mammals such as plateau pikas, Gansu pikas and even the forssorial rodent (Myosplax baileyi) showed respectively 44-108%, 124-137% and 39-53% higher in BMR than predicted values (Wang et al. 1979; 1991). The high cost of maintenance can partly interpret why BMR in winter-caught root voles was not significantly higher than that in other seasons.

Seasonal changes in serum leptin levels and regulation in body mass and thermogenesis

In seasonal small mammals, such as Brandt's voles (Li and Wang 2005a), collared lemmings (Nagy et al. 1995) and Siberian hamsters (Klingenspor et al. 2000), annual cycles of adiposity are correlated with seasonal changes in circulating leptin levels (Rousseau et al. 2003), suggesting that seasonal changes in circulating leptin concentrations may serve as a signal to regulate the body conditions. In the present study, seasonal differences in serum leptin levels did not disappear after adjusting for body mass, suggesting that the seasonal variations in serum leptin level could not be explained by the differences in body mass. In January, serum leptin levels were significantly lower in root voles, which was thought to act as a starvation signal (Flier 1998; Li and Wang 2005a) and thus can allow the animals to increase food intake to compensate the increased energy requirements in winter conditions; whereas in July, serum leptin levels were higher, conveying the animals a satiety signal to decrease the food intake to avoid getting fat (Berthoud 2005). It has been showed that when exposed to cold, energy intake was increased in root voles (Wang et al. 1996). Further, leptin levels were correlated with changes in body mass and body fat mass, which showed the lowest in winter and highest in summer and/or autumn, indicating that leptin worked as adiposity indicator and was involved in body mass regulation in root voles.

Although leptin was demonstrated to be able to affect energy expenditure, the available data were controversial. It was reported that leptin administration caused increases in oxygen consumption and UCP1 gene expression in BAT in rat and mice (Scarpace et al. 1997; Commins et al. 2001), whereas Abelenda et al. (2003) showed that leptin administration to cold-acclimated rats decreased UCP1 protein concentrations in BAT and reduced thermogenesis. Further, low serum leptin levels were found to be accompanied with increased UCP1 gene expression in rats exposed to cold (Bing et al. 1998) and similar variations were also found in seasonally acclimatized Brandt's voles (Li and Wang 2005a). Our data also showed a negative correlation between leptin concentration and UCP1 protein contents. These results indicated a potential involvement of leptin in the thermogenic capacity of BAT. However, pharmacological leptin administration needs to be conducted in further studies to determine whether food intake and energy expenditure are sensitive to exogenous leptin in root voles.

The physiological modification in the field is often hypothesized to allow an organism to make adjustments to changing environmental conditions (Bozinovic et al. 2003). From our study, we can suggest that the observed adjustments from molecular, cellular to organismal level of field-caught root voles are critically important for this species to successfully overcome the physiological challenges of the extreme environments in their habitat. In contrast, Nespolo et al. (2001) found that a fossorial rodent (Spalacopus cyanus), that escapes seasonal environmental extremes by burrowing, had a relatively low physiological plasticity. In summary, the observed seasonal regulations in serum leptin level and UCP1 protein content coupled with seasonal adjustments in other physiological performances in root voles may serve as an important means to survive the cold winter for this alpine rodent.

Acknowledgments We wish to thank Ying-Nian Li, Northwest Plateau Institute of Biology, the Chinese Academy of Sciences, for helping catch the animals. Thanks to Dr. Martin Klingenspor, Department of Biology, Philipps-University Marburg, Germany, for providing the hamster UCP1 antibody. Thanks to all the members of Animal Physiological Ecology Group, Institute of Zoology of the Chinese Academy of Sciences, for helping the experiments. This study was financially supported by the National Natural Science Foundation of China (No. 30430140 and No. 30170151) and the Chinese Academy of Sciences (No. KSCX2-SW-103) to DHW.

References

- Abelenda M, Ledesma A, Rial E, Puerta M (2003) Leptin administration to cold-acclimated rats reduces both food intake and brown adipose tissue thermogenesis. J Therml Biol 28:525–530
- Barbara C, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84:277–359
- Berthoud (2005) A new role for leptin as a direct satiety signal from the stomach. Am J Physiol Regul Integr Comp Physiol 288:796–797
- Bing C, Frankish HM, Pickavance L, Wang Q, Hopkins DFC, Stock MJ, Williams G. (1998) Hyperphagia in cold-exposed rats is accompanied by decreased plasma leptin but unchanged hypothalamic NPY. Am J Physiol Regul Integr Comp Physiol 274:R62–R68
- Bozinovic F, Gallardo PA, Visser RH, Cortés A (2003) Seasonal acclimatization in water flux rate, urine osmolality and kidney water channels in free-living degus: molecular mechanisms, physiological processes and ecological implications. J Exp Biol 206:2959–2966
- Bozinovic F, Bacigalupe LD, Vasquez RA, Visser GH, Veloso C, Kenagy G.J (2004) Cost of living in free-ranging degus (*Octodon degus*): seasonal dynamics of energy expenditure. Comp Biochem Physiol A 137:597–604

- Brunhoff CK, Galbreath E, Fedorov VB, Cook JA, Jaarola M (2003) Holarctic phylogeography of the root vole (*Microtus oeconomus*): implications for late Quaternary biogeography of high latitudes. Mol Ecol 12:957–968
- Buckley CA, Schneider JE (2003) Peptides that Regulate Food Intake Food hoarding is increased by food deprivation and decreased by leptin treatment in Syrian hamsters. Am J Physiol Regul Integr Comp Physiol 285:R1021–R1029
- Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84(1):277–359
- Commins SP, Watson PM, Frampton IC, Gettys TW (2001) Leptin selectively reduces white adipose tissue in mice via a UCP1-dependent mechanism in brown adipose tissue. Am J Physiol Endocrinol Metab 280:E372–E377
- Dijk G.V (2001) The role of leptin in the regulation of energy balance and adiposity. J Neuroendocrinol (13):913–921
- Feist DD, Feist CF (1986) Effect of cold, short day and melatonin on thermogenesis, body weight and reproductive organs in Alaskan red-backed voles. J Comp Physiol B 156:741–746
- Friedman JM, Halaas JL (1998) Leptin and the regulation of body weight in mammals. Nature 395:763–770
- Haim A (1996) Food and energy intake, non-shivering thermogenesis and daily rhythm of body temperature in the bushytailed gerbil *Sekeetamys calurus*: The role of photoperiod manipulations. J Therm Biol 21(1):37–42
- Heldmaier G (1971) Zitterfreie wärmebildung und körpergröße bei säugetieren. Z Vergl Physiologie 73:222–248
- Heldmaier G, Steinlechner S, Rafael J (1982) Nonshivering thermogenesisand cold resistance during seasonal acclimatization in the Djungarian hamster. J Comp Physiol B 149:1–9
- Janskey L (1973) Non-shievering thermogenesis and its thermoregulatory significance. Biol Rev 48:85–132
- Jefimow M, Wojciechowski M, Tegowska E (2004a) Seasonal changes in the thermoregulation of laboratory golden hamsters during acclimation to seminatural outdoor conditions. Comp Biochem Physiol A 139(3):379–388
- Jefimow M, Wojciechowski M, Tegowska E (2004b) Seasonal and daily changes in the capacity for nonshivering thermogenesis in the golden hamsters housed under semi-natural conditions. Comp Biochem Physiol A 137(2):297–309
- Jia XX, Sun RY (1986) Characteristics of the resting metabolic rate of the root vole at high altitude. Acta Zool Sinica 32: 280–287 (In Chinese with English summary)
- Johnson MS, Onorato DP, Gower BA, Nagy TR (2004) Weight change affects serum leptin and corticosterone in the collared lemming. Gen Comp Endocrinol 136(1):30–36
- Klause S, Heldmaier G, Ricquier D (1988) Seasonal acclimation of blank voles and wood mice: noshievering thermogenesis and thermogenic properties of brown adipose tissue mitochondria. J Comp Physiol B 158:157–164
- Klingenspor M, Dickopp A, Heldmaier G, Klaus S (1996) Short photoperiod reduces leptin gene expression in white and brown adipose tissue of Djungarian hamster. FEBS Letts 399:290–294
- Klingenspor M, Niggemann H, Heldmaier G. (2000) Modulation of leptin sensititivity by short photoperiod acclimation in the Djungarian hamster *Phodopus sungorus*. J Comp Physiol B 170:37–43
- Krauss S, Zhang CY, Lowell BB (2005) The mitochondrial uncoupling-protein homologues. Nat Rev Mol Cell Biol 6:248–261
- Kronfeld-Schor N, Haim A, Dayan T, Zisapel N, Klingenspor M, Heldmaier G. (2000) Seasonal thermogenic acclimation of diurnally and nocturnally active desert spiny mice. Physiol Biochem Zool 73(1):37–44

- Li QF, Sun RY, Huang CX, Wang ZK, Liu XT, Hou JJ (2001) Cold adaptive thermogenesis in small mammals from different geographical zones of China. Comp Biochem Physiol *A* 129:949–961
- Li XS (2005) Mechanism of body mass regulation and thermogenesis in Brandt's voles and Mongolian gerbils. Ph.D Dissertation. Institute of Zoology, the Chinese Academy of Sciences
- Li XS, Wang DH (2005a) Regulation of body weight and thermogenesis in seasonally acclimatized Brandt's voles (*Microtus brandti*). Horm Behav 48(3):321–328
- Li XS, Wang DH (2005b) Seasonal adjustments in body mass and thermogenesis in Mongolian gerbils (*Meriones unguiculatus*): the roles of short photoperiod and cold. J Comp Physiol B 175:593–600
- Li YN, Zhao XQ, Cao GM, Zhao L, Wang QX (2004) Analysis on climates and vegetation productivity background at Haibei Alpine Meadow Ecosystem Research Station. Plateau Metrol 23(4):558–567 (In Chinese with English summary)
- Lowry OH, Rosbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin-phenol reagents. J Biol Chem 193:265
- Lynch GR (1973) Seasonal changes in thermogenesis, organ weights, and body composition in the whited-footed mouse, *Peromyscus leucopus*. Oecologia (Berl) 13:363–376
- Nagy TR, Gower BA, Stetson MH (1995) Endocrine correlates of seasonal body mass dynamics in the collared lemming *Dicrostonyx groenlandicus*. Am Zool 35:246–258
- Nespolo RF, Bacigalupe LD, Rezende EL, Bozinovic F (2001) When nonshivering thermogenesis equals maximum metabolic rate: thermal acclimation and phenotypic plasticity of fossorial *Spalacopus cyanus* (Rodentia). Physiol Biochem Zool 74:325–332
- Nicholls DG, Locke RM (1984) Thermogenic mechanisms in brown fat. Physiol Rev 64:1–64
- Paracchini V, Pedotti P, Taioli E (2005) Genetics of leptin and obesity: a huge review. Am J Epidemiol 162(2):101–114
- Praun CV, Burkert M, Gessner M, Klingenspor M (2001) Tissue specific expression and cold-induced mRNA levels of uncoupling proteins in the Djungarian hamster. Physiol Biochem Zool 74(2):203–211
- Ricquier D, Bouilloud F (2000) The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. Biochem J 345:161–179
- Rousseau K, Atcha Z, Loudon ASI (2003) Leptin and seasonal mammals. J Neuroendo 15:409–414
- Scantlebury M, Oosthuizen MK, Speakman JR, Jackson CR, Bennett NC (2005) Seasonal energetics of the Hottentot golden mole at 1500 m altitude. Physiol Behav 84(5): 739–745

- Scarpace PJ, Matheny M, Pollock BH, Tumer N (1997) Leptin increases uncoupling protein expression and energy expenditure. Am J Physiol Endocrinol Metab 273:E226–E230
- Wang DH, Wang ZW (1989) Strategies for survival of small mammals in a cold environment I. Seasonal variations in the weight and structure of brown adipose tissue in Ochotona curzoniae and Microtus oeconomus. Acta Theriol Sinica 9:176–185 (In Chinese with English summary)
- Wang DH, Wang ZW (1996) Seasonal variations in thermogenesis and energy requirements of plateau pikas Ochotona curzoniae and root voles Microtus oeconomus. Acta Theriol 41(3):225–236
- Wang DH, Wang ZW (2000) Metabolism and thermoregulation in root vole (*Microtus oeconomus*) from the Qinghai-Tibetan Plateau. Mamm Biol 65:15–20
- Wang DH, Sun RY, Wang ZW (1996) Maximum energy assimilation rate in the root voles (*Microtus oeconomus*). Acta Zool Sinica 42(1):35–41 (In Chinese with English summary)
- Wang DH, Sun RY, Wang ZW, Liu JS (1999) Effects of temperature and photoperiod on thermogenesis in plateau pikas (Ochotona curzoniae) and root voles (Microtus oeconomus). J Comp Physiol B 169(1):77–83
- Wang DH, Wang ZW, Feng Y (1991) Strategies for survival of small mammals in a cold alpine environment. Thermoregulation of *Ochotona cansus* and adaptive convergence of small mammals to cold and high altitude. Alpine Meadow Ecosystem 3:125–137 (In Chinese with English summary)
- Wang ZW, Zeng JX, Han YC (1979) Studies on the metabolism rates of the mouse hare (*Ochotona curzoniae*) and the mole rat (*Myospalax fontanierii*). Acta Zool Sinica 25(1):75–85 (In Chinese with English summary)
- Wunder BA, Dobkin DS, Gettinger RT (1977) Shifts of thermogenesis in the prairie vole (*Microtus ochrogaster*): strategies for survival in a seasonal environment. Oecologia 29:11–26
- Zeng JX, Wang ZW, Han YC (1981) On the daily activity rhythm of five small mammals. Acta Theriol Sinica 1:189– 197 (In Chinese with English summary)
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and it's human homologue. Nature 372:425–432
- Zhang ZQ (2005) Seasonal changes in thermogenesis, energy budgets and immune function in Mongolian gerbils (*Meriones unguiculatus*). Ph.D Dissertation. Institute of Zoology, the Chinese Academy of Sciences
- Zhao ZJ, Wang DH (2005) Short photoperiod enhances thermogenic capacity in Brandt's voles. Physiol Behav 85(2):143– 149