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High body weight associated with impaired nonshivering thermogenesis but improved glucose tolerance in Mongolian gerbils (*Meriones unguiculatus*)

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ABSTRACT

Overweight and obesity correspond with metabolic syndromes, such as glucose intolerance and type 2 diabetes. The objective of this study was to determine whether decreased thermogenesis mass and glucose intolerance are directly related to changes in body mass in Mongolian gerbils. High body weight gerbils displayed increase in total body fat mass especially epididymal fat pad, and decrease in nonshivering thermogenesis, as indicated by depressed mitochondrial protein content and uncoupling protein-1 content in brown adipose tissue. No variations of sirtuin 1 and subunit IV of cytochrome oxidase expression were found in brown adipose tissue and skeletal muscle between the two groups. High body weight gerbils showed increased serum leptin and insulin concentrations but surprisingly increased glucose tolerance, suggesting a difference from other obese species in the regulation of glucose metabolism. Serum leptin levels were negatively correlated with UCP1 content in BAT and positively correlated with energy intake and insulin concentration. Our data suggest that leptin may be involved in thermogenesis regulation, insulin secretion and glucose metabolism in HBW gerbils.

1. Introduction

A current major public health challenge is the increasing prevalence of obesity and overweight and their associated pathologies, such as metabolic syndrome, insulin resistance and type 2 diabetes (Kahn and Flier, 2000; Stein and Colditz, 2004; Eckel et al., 2005; Flegal et al., 2010). Changes in life style in modern society, such as popularity of fast food and sedentary lifestyle, play a substantial role in the current epidemics of obesity (Jacobs, 2006; Bessesen, 2008). The reason leading to overweight or obesity is a prolonged imbalance between the levels of energy intake and expenditure (Hill, 2006). Fast food likely contributes to over consumption, while sedentary lifestyle reduces energy expenditure.

Mice and rats maintained under standard laboratory conditions are also sedentary and have continuous access to food. Compared with animals that are fed less and exercise more, they are relatively overweight and insulin resistant (Martin et al., 2010). Indeed, simply reducing daily food intake or increasing exercise has been shown to significantly reduce the risk of developing diseases such as type 2 diabetes and can extend lifespan in rats and mice (McCay et al., 1935; Goodrick, 1980; Martin et al., 2010). The assessment of obesity in wild animals is lacking, it is obvious that animals in captivity tend to maintain higher body weights and body fat mass than in the wild (Kirkwood, 1991; Schwitzer and Kaumanns, 2001). However, metabolic phenotype and glucose metabolism in response to body weight gain have not been systematically investigated in wild rodents living in a laboratory environment. Further study will provide more information about the prevalence of obesity.

Mongolian gerbil (*Meriones unguiculatus*) is distributed primarily in desert grasslands and agricultural fields of Inner Mongolia of China, Mongolia, and Russia (Wang et al., 2003; Liu et al., 2007). Thermogenesis and body weight regulation of Mongolian gerbils have been well investigated before (Li and Wang, 2005b; Zhao and Wang, 2006; Zhang and Wang, 2007b). High variability of body weight was also found in different studies of Mongolian gerbils (Stuermer et al., 2003). Our understanding of body weight regulation comes mainly from studies on mice and rats (Speakman et al., 2007). Individual differences in body weight in Mongolian gerbils may provide evidence for understanding the regulation of body weight and origin of the metabolic syndrome. In the present study, we hypothesize that body mass could affect thermogenesis and glucose tolerance in Mongolian gerbils. According to the hypothesis, we predict that higher body mass would be associated with decreased thermogenesis and glucose

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intolerance in Mongolian gerbils living in laboratory conditions. Parameters such as energy intake, resting metabolic rate (RMR), adaptive thermogenesis, fat deposition, serum leptin and insulin levels, and glucose tolerance were measured respectively.

2. Materials and methods

2.1. Animals and experimental design

Animal procedures were carried out in agreement with the Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences. Mongolian gerbils used in this study were from our laboratory colony live-trapped in Inner Mongolia and maintained at 23 ± 1 °C on a 16 h:8 h light:dark cycle (lights on at 04:00). Gerbils were individually housed in plastic cages ($30 \times 15 \times 20$ cm^3) with sawdust as bedding, food and water were available ad libitum. Commercial rat pellets (Beijing KeAo Feed Co.) were used as standard diet.

The experimental design has been described in detail by Xu et al. (2011). Briefly, 174 male gerbils born over a period of time in our lab (age: 9–16 months; body mass: 55.8–144.7 g) were individually housed and weighed, then the high (90.8–127.6 g, $n=16$) and low (60.5–77.7 g, $n=16$) body weight individuals were randomly selected from 26 heaviest (top 15%) and 26 lightest (bottom 15%) ones of all males, we defined the two groups as high body weight group (HBW) and low body weight group (LBW). We measured body mass, daily energy intake, RMR, and glucose tolerance of each gerbil. We also have used these animals to assess cellular and humoral immunity of gerbils with different body mass in another study (Xu et al., 2011). Following those measurements, animals were sacrificed by CO_2 overdose between 09:00 h and 11:00 h. The interscapular brown adipose tissue (IBAT) was immediately and carefully dissected, weighed and stored at −80 °C until assayed. Blood samples were collected, clotted for 1 h and centrifuged at 4 °C for 30 min at 4000 rpm; serum was then collected and stored at −80 °C until assayed.

2.2. Body mass and food intake

Body masses of Mongolian gerbils (± 0.1 g) were monitored using a digital balance (Sartorius, Goettingen, Germany). After dissection of IBAT, mesenteric fat pad, epididymal fat pad, retroperitoneal fat pad and subcutaneous fat pad were also dissected carefully and weighted (± 1 mg). The white fat pads were placed into the carcass and dried in an oven at 60 °C to constant mass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus. Fat free body mass was then calculated using final body mass minus total body fat in carcass.

Food intake was measured in metabolic cages for three consecutive days, after that the food residues and feces were collected, oven-dried at 60 °C to constant mass, and separated manually. Dry matter intake (DMI) was calculated from the difference between the food given and food residue. The caloric values of food and feces were determined by Parr1281 oxygen bomb calrimeter (Parr Instrument USA). Digestible energy intake (DEI) was then calculated as follows (Grodzinski and Wunder, 1975; Zhang and Wang, 2007a):

DEI (kJ/day)=Dry matter intake (g/day) × food gross energy (kJ/g) − dry feces mass (g/day) × feces gross energy (kJ/g).

2.3. Metabolic trials

Between 07:00 h and 20:00 h, resting metabolic rate (RMR) of gerbils was assessed at around 30 °C (within their thermal neutral zone) using a FOXBOX O_2 and CO_2 analyzer (Sable systems, NV, USA). Individual gerbils were placed in a metabolic chamber ($200 \times 130 \times 85$ mm^3) for 2 · hours. The flow rate of air (dried with anhydrous $CaSO_4$; W.A. Hammond Drierite Co., USA) was 600–800 ml min^{-1}. Gases leaving the chamber were dried (ND−2; Sable systems, NV, USA) and passed through the oxygen and carbon dioxide analyzer at approximately 100 ml min^{-1}. O_2 and CO_2 concentrations in the air were recorded as input concentration for 5 min before and after animal determination, and analyzer outputs were recorded every 6 s. The rate of oxygen consumption was calculated by the following equations (Hill, 1972; Li et al., 2010) and RMR was estimated from the stable lowest rate of oxygen consumption over 5 min.

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

where FR=flow rate (ml/min), Fi=input fractional concentration (%), Fe=excurrent fractional concentration (%).

2.4. Measurement of mitochondrial protein content in BAT

For measuring total mitochondrial protein content, BAT was homogenized as described previously (Zhao and Wang, 2005). Briefly, removed BAT was quickly homogenized with medium A. then the homogenate was centrifuged at 12096*g* for 10 min at 4 °C, and the precipitate was resuspended with medium B. after centrifuged at 500*g* for 10 min at 4 °C, the supernatant was then

Table 1
Body weight, body fat pad distribution, BAT functions and serum leptin level in Mongolian gerbils with low and high body weights.

Parameters	LBW	HBW	*P* value
Age (month)	17.0 ± 2.5	17.7 ± 2.3	$P=0.472$
Final body mass (g)	65.7 ± 5.8	106.0 ± 9.4	$P<0.001$
Fat free body mass (g)	57.9 ± 3.8	83.0 ± 6.8	$P<0.001$
Body fat pads mass (g)			
Mesenteric fat pad	0.8 ± 0.1	3.0 ± 0.3	$P=0.082$
Epididymal fat pad	1.0 ± 0.7	4.0 ± 0.7	$P<0.05$
Retroperitoneum fat pad	0.9 ± 0.6	2.9 ± 0.6	$P=0.096$
Subcutaneous fat pad	2.1 ± 1.0	5.3 ± 1.0	$P=0.084$
Total fat pad	4.5 ± 2.2	13.7 ± 2.2	$P<0.05$
Interscapular BAT mass	0.154 ± 0.066	0.513 ± 0.127	$P<0.05$
Total mitochondria protein in interscapular BAT (mg)	2.53 ± 0.16	2.99 ± 0.27	$P=0.155$
Total UCP1 in interscapular BAT (RU)	1.00 ± 0.11	0.84 ± 0.13	$P=0.373$
Serum leptin (ng/ml)	18.7 ± 3.4	31.8 ± 3.5	$P<0.01$

Values are means ± SE ($n=16$). Values for a specific parameter that share different superscripts are significantly different at $P<0.05$. Body fat pads were determined by a one-way ANCOVA with lean body mass as covariate followed by LSD *post-hoc* tests. Age, final body mass, fat free body mass, total mitochondria protein and UCP1 in interscapular BAT and serum leptin were analyzed by independent-samples *t*-test. LBW: low body weight; HBW: high body weight.

centrifuged at 8740*g* for 10 min at 4 °C, and the pellet was resuspended (1:1 w/v) with medium C. Mitochondrial and total protein concentrations of BAT were determined by Folin phenol method using bovine serum albumin as standard (Lowry et al., 1951). Total IBAT protein was lysed in RIPA buffer (1% Triton X-100, 158 mM NaCl, 5 mM EDTA, 10 mM Tris [pH 7.0], protease inhibitor cocktail [Sigma, St. Louis, Missouri, USA], 1 mM DTT, and 0.1% phenylmethylsulfonyl fluoride) (Bordone et al., 2006) and separated in a discontinuous SDS–polyacylamide gel (80 μg/lane, 10.0% running gel and 3% stacking gel). Uncoupling protein 1 (UCP1), cytochrome c oxidase 4 (COX4) and SIRT1 content in IBAT and muscle were measured by Western blotting. Primary antibodies used were as follows: rabbit anti-UCP1 (ab10983, Abcam, Cambridge, MA, USA), diluted 1:5,000; mouse anti-COX4 (sc-58348, Santa Cruz Biotechnology, Inc., CA, USA), diluted 1:500; rabbit anti-SIRT1 (sc-15404, Santa Cruz Biotechnology, Inc.), diluted 1:500; mouse anti-β-tubulin (E7, DSHB, Iowa City, Iowa, USA), diluted 1:2000. The secondary antibodies of goat anti-rabbit IgG (1:5000; ZSGB-BIO Co., Beijing, China) and goat anti-mouse IgG (1:5000; ZSGB-BIO Co., Beijing, China) were used correspondingly. Contents of target proteins were then calculated after being normalized with tubulin levels.

2.5. Serum leptin and insulin level assay

Serum leptin levels were measured by radioimmunoassay (RIA) with a ^{125}I multi-species kit (Cat. no. XL-85 K, Linco Research Inc. USA) (Li and Wang, 2005a; Zhang and Wang, 2007b). The lower and upper limits of the assay kit were 1 and 50 ng/ml and the intra- and inter-assay variations were $<3.6\%$ and 8.7%, respectively. Serum insulin levels were measured by radioimmunoassay (RIA) with a ^{125}I human kit (Atom HighTech Co., Ltd., Beijing, China) (Liu and Wang, 2011). The lower and upper limits of the assay kit were 5 and 160 ng/ml and the intra- and inter-assay variations were $<10\%$ and 15%, respectively. Serum leptin and insulin levels were determined in a single RIA and expressed as ng/ml.

2.6. Intraperitoneal glucose tolerance test

Intraperitoneal glucose (2 g/kg body weight) tolerance tests were conducted after fasting overnight. Blood samples were taken by tail venipuncture between 08:00 h and 11:00 h for glucose measurements by FreeStyle Mini Blood Meter (Abbott Diabetes Care Inc. Alameda, USA). Immediately before, 15, 30, 60 and 120 min after intraperitoneal glucose administration, blood glucose was collected and measured (Baur et al., 2006).

2.7. Statistical analyses

Data were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. To standardize body weight and body fat influences, group differences in white fat pad distributions, RMR and digestible energy intake were analyzed by a one-way analysis of covariance (ANCOVA) with fat free body mass as covariate followed by LSD *post-hoc* tests (Butler and Kozak, 2010). Group differences in other parameters (body mass; UCP1, SIRT1 and COX4 content; leptin and insulin concentrations) were analyzed by independent-samples *t*-test. Blood glucose tolerance was analyzed by repeated measures ANOVA followed by Bonferroni *post-hoc* tests. To detect possible associations of serum leptin with daily energy intake or insulin levels, we used Pearson correlation analyses. Results are presented as mean ± SE, and $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Body mass, body fat content and serum leptin levels

Age had no significant difference between HBW and LBW groups, but HBW gerbils exhibited both greater body weight ($t=19.489$, df=30, $P<0.001$, Table 1) and fat free body weight ($t=12.924$, df=30, $P<0.001$, Table 1) than LBW gerbils. Similar patterns of increased epididymal fat pad ($F_{1,29}=5.569$, $P<0.05$) and total body fat pad mass ($F_{1,29}=4.868$, $P<0.05$) were detected in HBW gerbils. Although mesenteric fat pad ($F_{1,29}=3.250$,

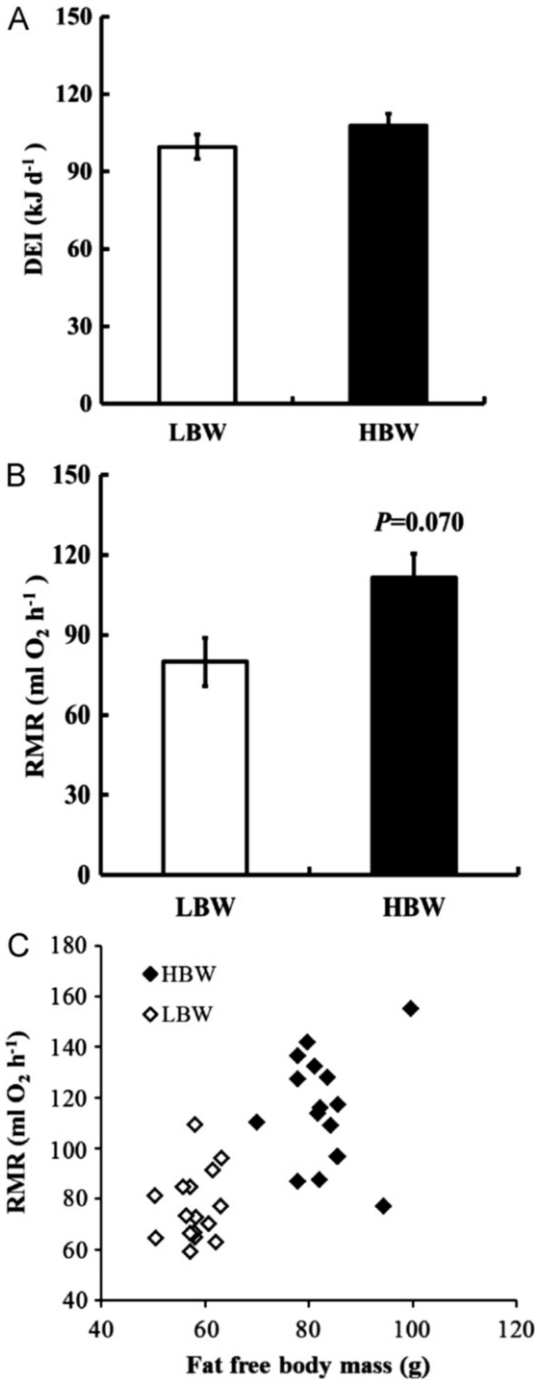

Fig. 1. Effects of body weight on daily energy intake (DEI) (A) and resting metabolic rate (RMR) (B) in Mongolian gerbils. HBW gerbils had similar levels of DEI and showed slight increases in their RMR compared to LBW group. Data are mean ± SE ($n=16$). One-way ANCOVA with fat free body mass as covariate.

$P=0.082$), retroperitoneal fat pad ($F_{1,29}=2.953$, $P=0.096$), and subcutaneous fat pad mass ($F_{1,29}=3.210$, $P=0.084$) slightly increased in HBM animals, there was no difference between the two groups (Table 1). Coupled with increased total body fat pad mass, there were parallel rise in serum leptin levels ($t=17.008$, df=30, $P<0.01$, Table 1). IBAT mass also shows significant increase in HBW gerbils ($F_{1,29}=6.897$, $P<0.05$).

3.2. Digestible energy intake and resting metabolic rate

Digestible energy intake (DEI) ($F_{1,29}=0.843$, $P=0.366$, Fig. 1A) had no significant difference between two groups. HBM gerbils slightly increased their resting metabolic rate (RMR) ($F_{1,29}=3.533$, $P=0.070$, Fig. 1B) when compared to LBW controls. We also provide a plot of individual RMR as a function of fat free mass, from which we can deduce the function between the two parameters: RMR=1.32 (fat free body mass) +2.48 ($R^2=0.48$) (Fig. 1C).

3.3. Protein content in BAT and muscle

Total mitochondria protein and UCP1 in interscapular BAT were not different in the two groups (Table 1). However, as compared to LBW animals, HBW group significantly decreased IBAT mitochondrial protein concentration per g tissue ($t=-12.085$, df=30, $P<0.001$, Fig. 2A). The same statistical result was also found on UCP1 expression per mg protein in IBAT ($t=-2.179$, df=30, $P<0.05$, Fig. 2B). No significant differences of BAT SIRT1 ($t=-0.187$, df=30, $P=0.853$, Fig. 2C) or COX4 ($t=1.601$, df=30, $P=0.120$, Fig. 2D) or muscle SIRT1 ($t=-0.394$, df=30, $P=0.696$, Fig. 2E) and COX4 ($t=1.641$, df=30, $P=0.111$, Fig. 2F) contents were found between LBW and HBW groups.

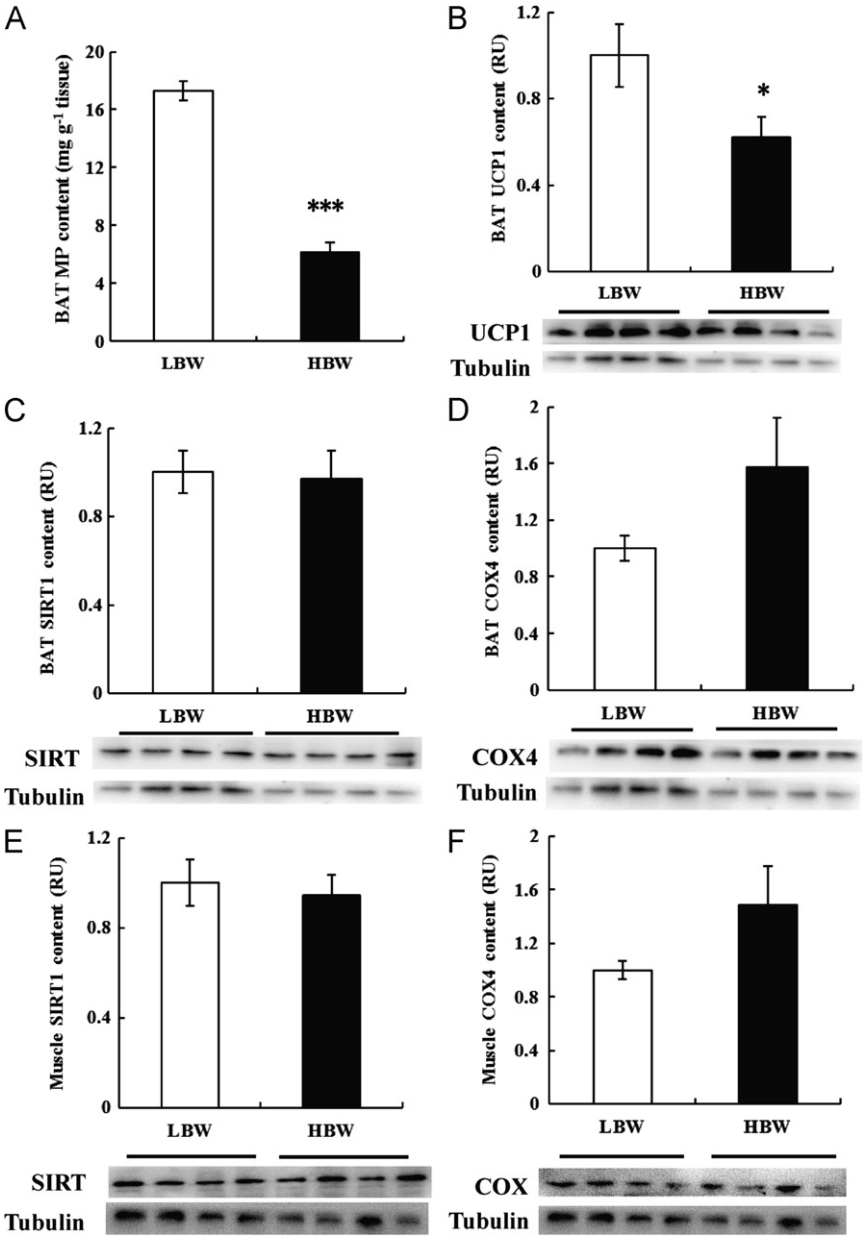

Fig. 2. Effects of body weight on protein contents of the brown adipose tissue and muscle in Mongolian gerbils. HBW gerbils had lower levels of BAT mitochondrial protein concentration (A) and UCP1 contents (B) than LBW gerbils. No significant differences of BAT SIRT1 (C) and COX4 (D) or muscle SIRT1 (E) and COX4 (F) were detected between the two groups. Data are mean ± SE ($n=16$). $^{*}p<0.05$, $^{***}p<0.001$, independent-samples t-test.

3.4. Serum insulin levels and intraperitoneal glucose tolerance test

HBW animals displayed ~14-fold higher insulin levels than in LBW controls ($t=15.142$, df=30, $P<0.001$, Fig. 3A). During intraperitoneal glucose tolerance tests, we found that blood glucose concentrations were significantly lower in HBW group compared with LBW group (group effect, $P<0.05$; time effect, $P<0.001$; group × time effect, $P<0.001$; repeated measures ANOVA, bonferroni correction; Fig. 3B).

3.5. Correlations of serum leptin levels with daily energy intake and insulin levels

There was a significant positive relationship between leptin and daily energy intake (total: $r=0.423$, $P<0.05$, Fig. 4A; HBW: $r=-0.177$, $P=0.511$; LBW: $r=0.050$, $P=0.853$) and insulin concentration ($r=0.487$, $P<0.01$, Fig. 4C; HBW: $r=0.496$, $P=0.051$; LBW: $r=0.383$, $P=0.143$). Serum leptin levels were negatively correlated with BAT UCP1 content ($r=-0.501$, $P<0.01$, Fig. 4B; HBW: $r=0.077$, $P=0.777$; LBW: $r=-0.490$, $P=0.054$).

4. Discussion

It has been previously reported that gerbils in captivity normally displayed high variability of body weight (39.42–95.16 g) (Stuermer et al., 2003), and body weight in male gerbils can reach 120 g and more (Kramer, 1964). Individual differences in body weight were also found in our laboratory gerbils (55.8–144.7 g in this study). Our present study showed that variability of body weight was associated with changes in metabolism parameters. Relative to their LBW counterparts, HBW gerbils possessed higher body fat pad mass, especially epididymal fat pad mass. Overweight gerbils had elevated levels of the energy regulatory hormones, leptin and insulin. Apart from HBW gerbils, overfed rats also exhibit elevated levels of body fat mass, glucose, insulin and leptin concentrations in comparison with reduced energy intake controls (Wang et al., 2001; Martin et al., 2010). Similar patterns of energy regulatory hormones and factors observed in overweight gerbils and overfed rodents indicated a relationship between them.

Our study showed that HBW Mongolian gerbils mainly employed decreased BAT mitochondrial protein concentration and UCP1 content to maintain their higher body mass. Patterns of subdued nonshivering thermogenesis (NST) in HBW gerbils are similar to wild gerbils in warm seasons or laboratory gerbils in long photoperiod (Zhao and Wang, 2006; Li and Wang, 2007; Zhang and Wang, 2007b). For non-hibernating temperate small mammals, low ambient temperatures in winter require increased BAT thermogenesis (Speakman, 2000), suggesting it is an important strategy for winter survival in field Mongolian gerbils. In contrast to BAT thermogenesis, RMR of gerbils slightly went up with increasing body mass. Rodent species from arid and semi-arid environments which have low values of RMR show high NST values (Haim and Izhaki, 1993; Chi and Wang, 2011). According to these results, we suggest that slightly higher RMR is a compensation for the low BAT thermogenesis in HBW gerbils.

Leptin plays fundamental roles in regulating energy intake, energy homeostasis and controlling body weight (Friedman and Halaas, 1998; Woods et al., 1998). Serum leptin concentration of gerbils was weakly negatively correlated with BAT UCP1 content in LBW gerbils of our study, suggesting that leptin may act as a factor in regulating nonshivering thermogenesis in gerbils with low body mass. It was confirmed that leptin could stimulate UCP1 expression in BAT (Scarpace et al., 1997; Friedman and Halaas, 1998; Scarpace and Matheny, 1998). In the present study, however, increased serum leptin levels were accompanied by a lower UCP1 content in LBW gerbils. It has been proposed that resistance to the biological effects of leptin is commonly associated with obesity (Frederich et al., 1995), our results also indicated that increase in leptin levels in HBW gerbils does not promote UCP1 expression, suggesting that HBW gerbils develop resistance to leptin. However, the role of leptin in thermogenesis in gerbils with different body masses needs to be further validated.

The association of obesity, particularly abdominal obesity, with increased mortality from insulin resistance has been recognized for decades (Kahn and Flier, 2000; Westphal, 2008). When the pancreas is incapable of releasing enough insulin to prevent insulin resistance, impaired glucose tolerance develops (Attallah et al., 2006). A surprising result from this study was that HBW gerbils associated with increased body fat mass had improved glucose tolerance, suggesting a difference from other obese species in the regulation of glucose metabolism. Several observations suggest that mitochondrial dysfunction in classic insulin target tissues such as skeletal muscle and fat may be a central cause of insulin resistance and glucose tolerance (Kelley et al., 2002; Barazzoni, 2004; Kim et al., 2008). Sirtuins (Elliott and Jirousek, 2008) and cytochrome c oxidase (COX) (Heldmaier and Buchberger, 1985) are two important factors in regulating mitochondrial activity and function. There were no significant differences in protein expression of SIRT1 and COX4, implying mitochondrial function is normal in skeletal muscle and BAT of HBW gerbils, it could be attributed to unimpaired glucose tolerance. Leptin is also expected to regulate insulin secretion and insulin action (Barzilai et al., 1997; Ookuma et al., 1998). Positive correlation between leptin and insulin concentration

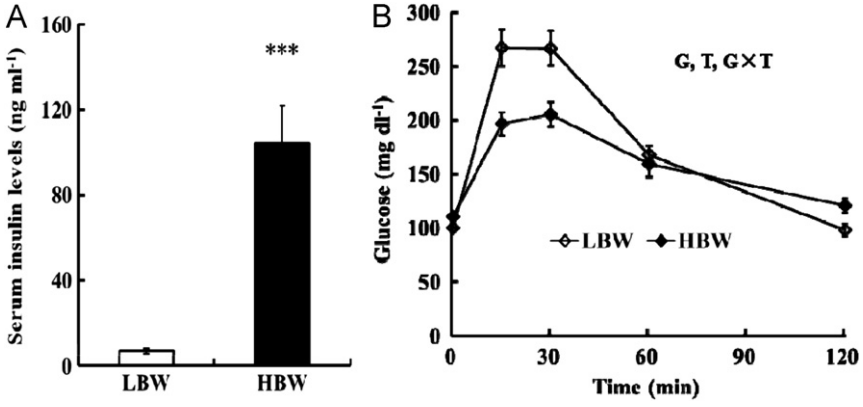

Fig. 3. Serum insulin concentration and glucose tolerance test in LBW and HBW animals. Serum insulin levels increased significantly in HBW gerbils, while blood glucose concentrations were significantly lower in HBW group compared with LBW group. Data are mean ± SE ($n=16$). *** $p<0.001$. G: group effect, $P<0.05$; T: time effect, $P<0.001$; G × T: group × time effect, $P<0.001$, repeated measures ANOVA.

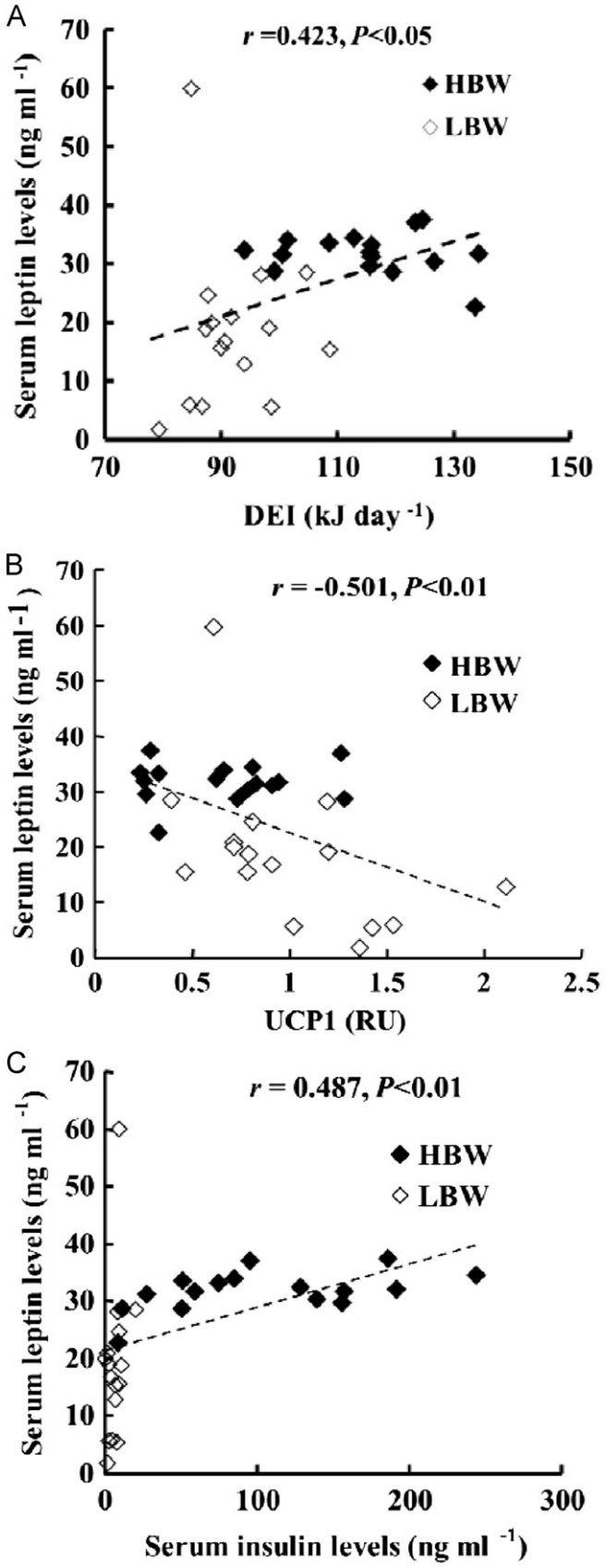

Fig. 4. Correlations of serum leptin concentration with BAT UCP1 content (A), daily energy intake (B) and insulin levels (C) in LBW and HBW gerbils. Serum leptin levels were negatively correlated with DEI and BAT UCP1, while positively correlated with insulin levels.

in HBW gerbils suggested possibility of leptin regulation in insulin secretion and glucose tolerance. However, it seems that small increase in leptin level could not explain the great insulin variation between different groups. There must be other mechanisms which can accelerate the secretion of insulin. Further study on the decisive factors of insulin secretion in HBW gerbils will give us further information on the cause of glucose intolerance.

Overall, HBW gerbils displayed decreased NST but dramatically increased insulin levels and improved glucose tolerance, suggesting it is an interesting model for studying the relationship between thermogenesis and glucose tolerance. Gerbils increase serum leptin and insulin concentrations in response to weight gain, suggesting these factors may participate in improving the glucose tolerance.

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