Seasonal Changes of Body Mass and Energy Budget in Striped Hamsters: The Role of Leptin

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ABSTRACT

Proper adjustments of physiology and behavior are required for small mammals to cope with seasonal climate change. The aim of this study was to examine the role of leptin in the regulation of body mass and energy budget in striped hamsters. We first investigated seasonal changes in body mass, energy budget, and serum leptin levels in hamsters acclimated to outdoor natural daylight and ambient temperature. Then we assessed the effect of leptin administration on energy budget, serum lipoprotein lipase (LPL) and hepatic lipase (HL) activities, and gene expression of uncoupling protein 1 (UCP1) in brown adipose tissue and of hypothalamic neuropeptides associated with the regulation of energy balance in hamsters maintained at 21° and 5°C. Hamsters showed constant body mass throughout the four seasons but significantly increased food intake and thermogenesis in winter, compared to summer. Minimum body fat was observed in winter, and minimum serum leptin was found in autumn. Hamsters housed at 5°C showed higher energy intake, upregulated gene expression of UCP1 and hormone-sensitive lipase, and lower fat content and LPL and HL activity than the animals maintained at 21°C. Leptin administration had no effect on energy intake but increased maximal thermogenic capacity, as indicated by upregulated UCP1 gene expression at both 21° and 5°C. Body fat and activity of LPL and HL were decreased in hamsters treated with leptin. The results suggest that leptin plays an important role in the seasonal regulation of thermogenic capacity and body composition in striped hamsters. Leptin may be involved in increasing maximal thermogenesis in the cold rather than acting as a starvation signal to increase energy intake.

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Introduction

Proper adjustments of both physiology and behavior are required for small mammals living in a temperate zone to cope with considerable changes in seasonal environment (Bartness and Wade 1985; Mercer 1998; Klingenspor et al. 2000; Concannon et al. 2001; Bartness et al. 2002; Jefimow et al. 2004). Physiological regulation associated with energy budget and thermoregulatory mechanisms are important for animals living through hot summers and cold winters (Zhao et al. 2010*a*, 2010*b*). Many small mammals usually increase energy intake and the energy spent for thermogenesis but decrease body mass in response to cold winters and/or winter-like conditions (Jefimow 2007), exhibiting considerable seasonal changes in energy budget and body and/or fat mass (Bartness and Wade 1985; Klingenspor et al. 2000; Bartness et al. 2002; Li and Wang 2005*a*, 2005*b*; Wang et al. 2006*a*, 2006*b*). The interaction of environment factors affecting energy budget and body mass makes seasonal rodents attractive as models for investigations of the mechanisms underlying energy balance (Rousseau et al. 2003).

Actually, many mammalian species undergoing seasonal changes in body mass/fatness show concomitant fluctuations in circulating leptin, a protein hormone produced and secreted by white adipose tissue (WAT; Zhang et al. 1994; Friedman and Halaas 1998; Concannon et al. 2001; Bartness et al. 2002). Leptin levels increased with accretion of adipose tissue mass and decreased when adipose mass was lost (Maffei et al. 1995; Friedman 2011; Zhao et al. 2013), indicating that leptin could play an important role in the regulation of energy budget and body mass/fatness in seasonal mammals (Rousseau et al. 2003). So far, many studies performed on laboratory animals, and even on humans, have suggested that leptin likely has the role of decreasing energy intake and increasing energy expenditure (Friedman and Halaas 1998; Woods et al. 1998; Concannon et al. 2001; Mercer and Speakman 2001; Bartness et al. 2002). Notable orexigenic peptides, including neuropeptide Y (NPY) and agouti-related peptide (AgRP), and the anorexigenic neuropeptides pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) in the hypothalamic arcuate nucleus may mediate anorexigenic leptin action by targeting a long-form leptin receptor (Ob-Rb; Friedman and Halaas 1998; Flier and Maratos-Flier 1998; Woods et al. 1998;

Ahima et al. 2000; Mercer and Speakman 2001; Zhang et al. 2012). It had been also found that administering leptin to obese mice significantly lowered lipoprotein lipase (LPL; EC 3.1.1.34) activity relative to that in lean mice, suggesting that leptin might stimulate lipolysis and consequently attenuate fat accumulation by decreasing LPL mass and activity (Picard et al. 1998; Hikita et al. 2000).

However, results from field animals under a natural environment may not be always consistent with those from laboratory animals maintained at room conditions. For example, in some seasonal animals, the increased body and/or fat mass was in parallel with increased leptin levels but the animals displayed decreased energy intake and expenditure in summer, suggesting that leptin may inhibit energy intake rather than stimulate energy expenditure (Klingenspor et al. 2000; Li and Wang 2005*a*, 2005*b*; Wang et al. 2006*a*, 2006*b*). When acclimated to cold winters or winter-like conditions, these animals exhibited significant reductions of body fat and leptin levels but great elevations of food intake and the energy expenditure associated with thermogenesis, implying that leptin might function in different ways in summer and winter. It might also suggest that the role of leptin is possibly associated with seasonal environment change, including photoperiod and temperature.

The striped hamster (*Cricetulus barabensis* Pallas, 1773) is a principal rodent in northern China and is also distributed in Russia, Mongolia, and Korea (Zhang and Wang 1998; Song and Wang 2003). The climate is arid and characterized by warm and dry summers and cold winters (Zhang and Wang 1998; Zhao et al. 2010*a*, 2010*b*; Zhao and Cao 2009). Striped hamsters do not show daily torpor and hibernation but develop good adaptive strategies to cope with changes in the seasonal environment, including seasonal enhancement in food intake and thermogenesis but excluding seasonal changes in body mass (Zhao et al. 2010*a*, 2010*b*). After being acclimated to a consecutive decrease in ambient temperature from 23° to −23°C, striped hamsters significantly increased energy intake and the mobilization of fat storage, which was not accompanied by a reduction in serum leptin levels (Zhao 2011). This suggests that the seasonal patterns of energy budget and body mass in striped hamsters are inconsistent with those in other rodents, which show seasonal increases in energy intake but decreases in body mass. It is obvious that striped hamsters must adjust their energy intake to balance energy expenditure and consequently maintain a constant body mass over four seasons, in which leptin may be involved. However, the roles of leptin in seasonal adjustments of energy intake and expenditure remain uncertain.

In this study, we investigated seasonal changes in body mass, fatness, energy budget, and serum leptin levels in striped hamsters acclimated to a seasonal environment. We also assessed the effect of leptin administration on energy intake and serum LPL and hepatic lipase (HL) activity as well as on expression of the genes encoding neuropeptides (NPY, AgRP, POMC, and CART) and the genes of WAT hormone-sensitive lipase (HSL; EC 3.1.1.3) and uncoupling protein 1 (UCP1) of brown adipose tissue (BAT). We hypothesized that leptin might have no role in the regulation of NPY/AgRP and POMC/CART gene expression, suggesting that leptin might not be involved in the control of energy intake in hamsters. This hypothesis could predict that leptin likely played an important role in increasing maximal thermogenesis and mobilization of fat reservoir.

Material and Methods

Animals

Striped hamsters were obtained from our laboratory breeding colony, which started with animals trapped from farmland at the center of Hebei Province (115°13′E, 38°12′S) in the North China Plain. This breeding colony was maintained under a 12L : 12D (lights on at 0800 hours) photoperiod, and room temperature was kept at 21° ± 1°C. Food (standard rodent chow; produced by Beijing KeAo Feed, Beijing) and water were provided ad lib.

Experiment 1 was designed to examine seasonal changes in body mass, energy budget, and thermogenesis. We transferred 52 male hamsters (3.5–4.5 mo old) from the laboratory to separate outdoor enclosures in mid-April and held them individually in plastic cages (29 cm × 18 cm × 16 cm) with fresh sawdust bedding. After 1 mo of adaptation to the outdoor enclosure, body mass and food intake were measured at 15-d intervals. Energy intake, basal metabolic rate (BMR), nonshivering thermogenesis (NST), body composition, and serum leptin levels were measured at the end of July (daily minimal and maximal temperatures were 24° and 33°C, respectively; 14.5L : 9.5D) and in early November (−4° and 7°C; 10.5L : 13.5D), mid-January (−11° and −2°C; 9.5L : 14.5D), and April (9°C and 17°C; 13L : 11D), hereafter referred to as "summer," "autumn," "winter," and "spring," respectively.

Food Intake, Gross Energy Intake (GEI), and Digestibility

Food intake was calculated as the amount of food missing from the hopper each day, subtracting the orts mixed into bedding. Food was provided quantitatively, and food residue and sawdust bedding mixed with feces were collected from each animal over 2 d. After they were dried in an oven at 60°C to constant mass, food residues and feces were sorted out manually (Liu et al. 2003). Gross energy contents of the diet and feces were determined by a Parr 1281 oxygen bomb calorimeter (Parr Instrument, Moline, IL). Dry-matter intake (DMI, in g/d), GEI (in kJ/d), digestive energy intake (DEI, in kJ/d), and digestive efficiency (%) were calculated as follows (Grodzinski and Wunder 1975; Liu et al. 2003; Zhao et al. 2013):

$$\text{DMI} = \text{food intake (g/d)} \times \text{dry-matter content of food (\%)},$$

$$\text{GEI} = \text{DMI} \times \text{energy content of food (kJ/g)},$$

$$\text{DEI} = \text{GEI} - (\text{dry feces mass (g/d)} \times \text{energy content of dry feces (kJ/g)}),$$

$$\text{digestive efficiency} = \frac{\text{DEI}}{\text{GEI}} \times 100.$$

BMR and NST

BMR and NST were quantified as the rate of oxygen consumption in an open-flow respirometry system (Sable System, Las Vegas, NV), as described previously (Zhao 2012). Briefly, animals were fasted for 4 h before being transferred into the chamber. After 1 h of adaptation to the chamber, BMR was measured for 2.5 h at 29° ± 0.5°C (within the thermal neutral zone of this species; Zhao et al. 2010*a*). Oxygen consumption was calculated using the equation $\dot{V}O_2 = FR(Fi_2 - Fe_2)/[1 - Fi_2 \times (1 - RQ)]$, where FR is the flow rate, Fi_2 is the input fractional concentration of O_2 to the chamber, Fe_2 is the excurrent fractional concentration of O_2 from the chamber, and RQ is the respiratory quotient (Arch et al. 2006). Here, RQ was assumed to be 0.85 (Withers 1977; Chi and Wang 2011; Zhao 2012). BMR was calculated from the lowest rate of oxygen consumption over 5 min.

Maximum NST (NST_{max}) was measured after BMR and was quantified as the maximal rate of oxygen consumption induced by subcutaneous injection of norepinephrine (NE; Shanghai Harvest Pharmaceutical) at 25° ± 1°C. A mass-dependent dosage of NE was calculated according to the equation NE (mg/kg) = $6.6M_b^{-0.458}$ (g; Heldmaier 1971), where M_b is body mass. NST_{max} was measured for another 1 h. The highest rate of oxygen consumption over 5 min was taken to calculate NST_{max}. NST was calculated as NST_{max} − BMR. BMR, NST_{max}, and NST were corrected to standard temperature and air pressure conditions and expressed as milliliters of O_2 per hour (Zhao 2012). All measurements were made between 0900 and 1700 hours. The time is appropriate for the measurements because these animals are nocturnal.

Serum Leptin Levels

Animals were euthanized by decapitation between 0900 and 1100 hours. Trunk blood was collected for serum leptin measurements. Serum leptin levels were quantified by radioimmunoassay with the Linco ^{125}I Multispecies Kit (Linco Research, St. Charles, MO), following the standard kit instructions. 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.

Body Composition and Body Fat Content

After blood was collected, the gastrointestinal (GI) tract (stomach, small and large intestines, and cecum) were separated and weighed without their contents (to 1 mg). Liver, heart, lung, spleen, and kidneys were also removed and weighed (to 1 mg). The remaining carcass was weighed (to 1 mg) to determine wet mass, dried in an oven at 60°C for 10 d to constant mass, and reweighed (to 1 mg) to determine dry mass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus (Zhao and Wang 2006).

Experiment 2 was designed to check the effects of leptin administration in cold-acclimated animals. Twenty-four ham-

Table 1: Gene-specific primer sequences used for real-time RT-QPCR analysis

Gene	Primers (5′ to 3′)	Size (bp)
NPY (forward)	ACCCTCGCTCTGTCCCTG	186
NPY (reverse)	AATCAGTGTCTCAGGGCTA	186
AgRP (forward)	TGTTCCCAGAGTTCCCAGGTC	227
AgRP (reverse)	ATTGAAGAAGCGGCAGTAGCAC	227
POMC (forward)	GGTGGGCAAGAAGCGACG	205
POMC (reverse)	CTTGTCCTTGGGCGGGCT	205
CART (forward)	TACCTTTGCTGGGTGCCG	260
CART (reverse)	AAGTTCCTCGGGGACAGT	260
Ob-Rb (forward)	CAGTGTCGATACAGCTTGGA	200
Ob-Rb (reverse)	TTGCATATTTAACTGAGGGT	200
UCP1 (forward)	GGGACCATCACCACCCTGGCAAAAA	330
UCP1 (reverse)	GGCTTTCTGTTGTGGCTAT	330
HSL (forward)	CACTACAAACGCAACGAG	224
HSL (reverse)	CTGAGCAGGCGGCTTACC	224
Actin (forward)	AAAGACCTCTATGCCAACA	196
Actin (reverse)	ACATCTGCTGGAAGGTGG	196

Note. RT-QPCR, reverse transcriptase quantitative polymerase chain reaction; NPY, neuropeptide Y; AgRP, agouti-related protein; POMC, pro-opiomelanocortin; CART, cocaine- and amphetamine-regulated transcript; Ob-Rb, long form of the leptin receptor; UCP1, uncoupling protein 1; HSL, hormone-sensitive lipase (EC 3.1.1.3).

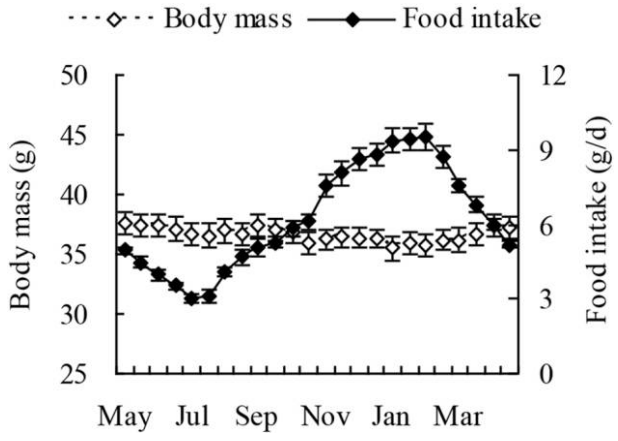

Figure 1. Seasonal changes in body mass (open symbols) and food intake (filled symbols) in striped hamsters. Body mass did not differ during the course of seasonal acclimation, while food intake decreased significantly from May to a minimum in July and thereafter increased significantly, reaching a maximum in February. Data are means ± SEM.

sters were randomly assigned into one of four groups ($n = 6$ for each group): in the 21°C–phosphate-buffered saline (PBS) and 21°C-leptin groups, hamsters were maintained at 21°C and treated with PBS and leptin, respectively; in the 5°C-PBS and 5°C-leptin groups, hamsters were maintained at 5°C and treated with PBS and leptin, respectively. On day 17 of cold exposure, an osmotic minipump (Alzet model 1007D; capacity, 100 μL; release rate, 0.5 μL/h; duration, 7 d; Durect, Cupertino, CA) filled with either recombinant murine leptin (100 μg dissolved in 100 μL PBS; PeproTech, London) or PBS only was inserted subcutaneously for 7 d. Body mass and food intake were measured daily. GEI, DEI, and digestibility were determined over the last 2 d of this experiment. After this experiment, animals were euthanized by decapitation between 0900 and 1100 hours, and serum was sampled. Serum LPL and HL activities were measured with commercial kits (Jiancheng Bioengineering Institute, Nanjing, China) following manufacturer's instructions. Body fat content and body composition were determined as described above.

Real-Time Reverse Transcriptase Quantitative Polymerase Chain Reaction (QPCR) Analysis

The hypothalamus, interscapular BAT, and subcutaneous WAT were removed quickly and stored in liquid nitrogen immediately. Total RNA was isolated from the hypothalamus, BAT, and WAT with a Trizol kit (TAKARA, Dalian, China) according to the manufacturer's instructions. RNA concentration and purity were determined by A260 and A280 optical density. The complementary DNAs were synthesized from 2 μg of total RNA in a final reaction volume of 20 μL with avian myeloblastosis virus (AMV) reverse transcriptase (TAKARA) using random primer Oligo $(dT)_{18}$. Then, 2 μL of the reverse transcription reaction was used as a template for the subsequent PCR reaction using gene-specific primers (table 1). QPCR was performed with the Mx3000P Real-Time QPCR system (Stratagene, La Jolla, CA), and reactions were done with the SYBR Green PCR master mix kit, following manufacturer's instructions under user-defined thermal cycling conditions (95°C for 30 s and 40 cycles at 95°C for 5 s, 55°C for 30 s, and 72°C for 30 s), followed by thermal denaturation curves. PCR fragments were cloned and sequenced to verify the gene-specific primers for the eight genes (NPY, AgRP, POMC, CART, UCP1, Ob-Rb, HSL, and actin; table 1). All samples were quantified for relative quantity of gene expression with actin expression as an internal standard.

Statistics

Data were expressed as mean ± SE and analyzed with SPSS 13.0 statistic software. For experiment 1, changes in body mass and food intake over a year were tested with repeated-measures ANOVA. Differences in BMR, NST, body fat, leptin levels, GEI, and digestibility, as well as body composition, were assessed by one-way ANOVA or ANCOVA with body mass as a covariates,

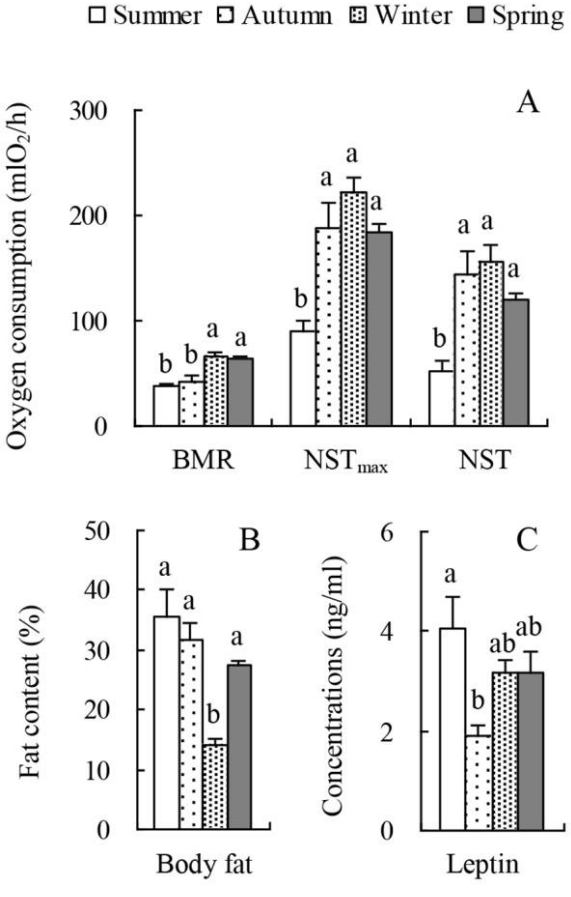

Figure 2. Seasonal changes in basal metabolic rate (BMR), maximum and ordinary nonshivering thermogenesis (NST_{max} and NST, respectively; *A*), body fat content (*B*), and serum leptin concentration (*C*) in striped hamsters. Data are means ± SEM. Different letters above the columns indicate significant differences between the seasons ($P < 0.05$).

Table 2: Seasonal changes in energy intake and digestibility in striped hamsters

	Summer ($n = 6$)	Autumn ($n = 6$)	Winter ($n = 8$)	Spring ($n = 6$)	P
DMI (g/d)	$3.9 \pm .4^{C}$	$5.6 \pm .2^{B}$	$8.6 \pm .3^{A}$	$4.1 \pm .2^{C}$	<.01
GEI (kJ/d)	68.3 ± 7.1^{C}	98.9 ± 3.8^{B}	152.1 ± 5.5^{A}	71.3 ± 4.3^{C}	<.01
Feces (g/d)	$.86 \pm .10^{C}$	$1.26 \pm .05^{B}$	$1.90 \pm .07^{A}$	$.88 \pm .04^{C}$	<.01
GE of feces (kJ/d)	13.5 ± 1.6^{C}	$19.7 \pm .7^{B}$	29.8 ± 1.0^{A}	$13.9 \pm .6^{C}$	<.01
DEI (kJ/d)	54.8 ± 5.6^{C}	79.2 ± 3.2^{B}	122.2 ± 4.6^{A}	57.4 ± 3.6^{C}	<.01
Digestibility (%)	$80.3 \pm .5$	$80.1 \pm .5$	$80.4 \pm .4$	$80.4 \pm .5$	NS

Note. DMI, dry matter intake; GEI, gross energy intake; GE, gross energy; DEI, digestive energy intake. Data are means ± SEM. Different letters in the same row indicate significant differences between the seasons ($P < 0.05$). $P < .01$ indicates a significant seasonal change; NS indicates a nonsignificant difference ($P > 0.05$).

followed by Tukey's HSD post hoc tests where required. For experiment 2, the effects of temperature and leptin on all parameters were examined with two-way ANOVA or ANCOVA (temperature × leptin) with body mass as a covariate, followed by Tukey's HSD post hoc tests where appropriate. Pearson's correlation was performed to examine relationships between fat content and LPL and HL activity. Significance was set at $P < 0.05$.

Results

Experiment 1

Body Mass and Food Intake. Body mass did not differ ($F_{23, 138} = 0.38$, $P > 0.05$; fig. 1), while food intake changed significantly over the four seasons ($F_{23, 161} = 78.91$, $P < 0.001$; fig. 1). Food intake averaged 5.0 ± 0.1 g/d in May and decreased significantly from May to a minimum in July (post hoc: $P < 0.05$). After that, food intake increased to a maximum in February, representing a 241% increase over intake in July (post hoc: $P < 0.05$; fig. 1).

BMR and NST. There was significant seasonal change in BMR, which was higher in winter and spring than in summer and autumn ($F_{4, 21} = 15.06$, $P < 0.01$; fig. 2*A*). NST_{max} and NST were also seasonally different, significantly higher in autumn, winter, and spring than in summer (NST_{max}: $F_{4, 21} = 11.04$, $P < 0.01$; NST: $F_{4, 21} = 7.54$, $P < 0.01$; fig. 2*A*).

Energy Intake and Digestibility. Striped hamsters showed significant change in DMI, which was higher in autumn and winter than in summer and spring ($F_{3, 21} = 73.44$, $P < 0.01$; table 2). There were also significant seasonal changes in GEI and DEI, which reached their maximum in winter and were higher by 122.8%, and 123.2%, respectively, than those in summer (GEI: $F_{3, 21} = 73.44$, $P < 0.01$; DEI: $F_{3, 21} = 66.54$, $P < 0.01$; post hoc: $P < 0.05$; table 2). Similarly, mass and gross energy (GE) of feces were at their maximum in winter and their minimum in summer (table 2). However, no seasonal change in digestibility was observed ($F_{3, 22} = 0.13$, $P > 0.05$).

Body Composition. Carcass mass differed between the four seasons, and it was lower by 17.0% in winter than in summer (table 3). There were seasonal changes in masses of liver, lung, and kidneys, and the maximum masses were observed in winter

Table 3: Seasonal changes in body composition in striped hamsters

	Summer ($n = 6$)	Autumn ($n = 6$)	Winter ($n = 8$)	Spring ($n = 6$)	P
Body mass (g)	37.8 ± 2.7	36.5 ± 1.4	$36.7 \pm .5$	37.6 ± 1.2	NS
Carcass mass (g)	29.50 ± 2.24^{A}	$25.96 \pm .87^{AB}$	$24.52 \pm .32^{B}$	$28.65 \pm .79^{AB}$	<.05
BAT (g)	$.109 \pm .010^{B}$	$.166 \pm .015^{A}$	$.111 \pm .007^{B}$	$.104 \pm .011^{B}$	<.01
Liver (g)	$1.325 \pm .132^{B}$	$1.695 \pm .060^{A}$	$1.964 \pm .069^{A}$	$1.678 \pm .060^{A}$	<.01
Heart (g)	$.180 \pm .011$	$.196 \pm .011$	$.233 \pm .010$	$.201 \pm .015$	.07
Lung (g)	$.244 \pm .010$	$.215 \pm .012$	$.280 \pm .022$	$.220 \pm .015$	<.05
Spleen (g)	$.035 \pm .005$	$.037 \pm .003$	$.032 \pm .003$	$.038 \pm .004$	NS
Kidneys (g)	$.363 \pm .029^{B}$	$.483 \pm .035^{A}$	$.530 \pm .024^{A}$	$.467 \pm .025^{AB}$	<.01
Stomach (g)	$.326 \pm .016^{B}$	$.354 \pm .038^{AB}$	$.437 \pm .015^{A}$	$.340 \pm .035^{AB}$	<.01
SI (g)	$.373 \pm .016^{B}$	$.395 \pm .023^{B}$	$.746 \pm .032^{A}$	$.377 \pm .018^{B}$	<.01
LI (g)	$.191 \pm .021^{B}$	$.282 \pm .024^{AB}$	$.291 \pm .025^{A}$	$.276 \pm .020^{AB}$	<.05
Cecum (g)	$.148 \pm .006^{B}$	$.179 \pm .016^{B}$	$.246 \pm .014^{A}$	$.171 \pm .013^{B}$	<.01

Note. BAT, brown adipose tissue; SI, small intestine; LI, large intestine. Data are means ± SEM. Different letters in the same row indicate significant differences between the seasons ($P < 0.05$); $P < 0.01$ and $P < 0.05$ indicate significant seasonal changes; NS indicates a nonsignificant difference ($P > 0.05$).

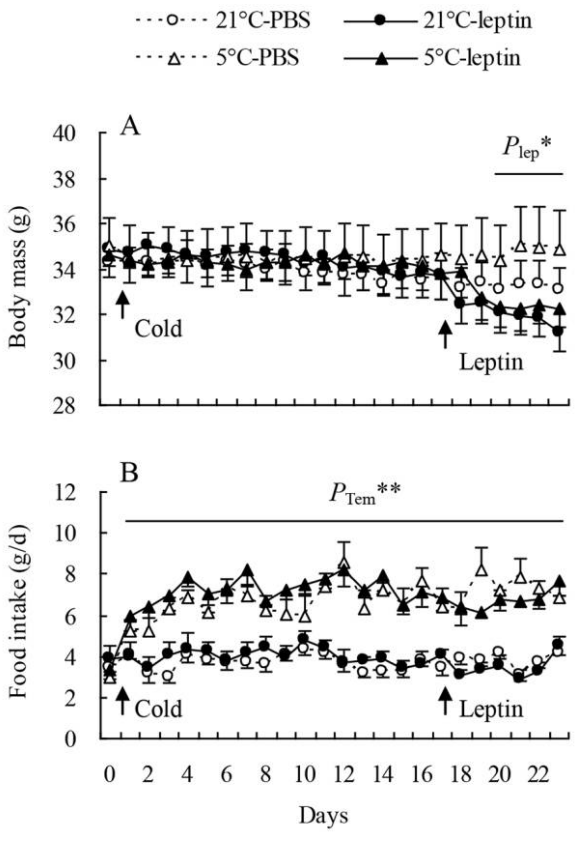

Figure 3. Effect of temperature and leptin supplementation on body mass (*A*) and food intake (*B*) in striped hamsters. "P_{Tem}**" indicates a significant effect of temperature ($P < 0.05$); "P_{lep}*" indicates a significant effect of leptin administration ($P < 0.01$). PBS, phosphate-buffered saline.

(table 3). Masses of stomach, small and large intestines, and cecum were also seasonally different, higher by 34.0%, 99.8%, 52.5%, and 66.5%, respectively, in winter than in summer (table 3).

Body Fat Content and Serum Leptin Concentration. There was significant seasonal change in body fat content, which was lower in autumn than in the other three seasons ($F_{3, 22} = 13.47$, $P < 0.01$; post hoc: $P < 0.05$; fig. 2*B*). Serum leptin levels showed significant seasonal changes and were highest in summer and lowest in autumn ($F_{3, 22} = 4.70$, $P < 0.05$; post hoc: $P < 0.05$; fig. 2*C*).

Experiment 2

Body Mass. There was no difference in body mass between the four groups before the experiment (day 0; temperature: $F_{1, 12} = 0.06$, $P > 0.05$; leptin: $F_{1, 12} = 0.03$, $P > 0.05$; post hoc: $P > 0.05$; fig. 3*A*). Body mass was not affected by temperature throughout the experiment (day 23; $F_{1, 12} = 2.34$, $P > 0.05$). Leptin supplementation had a significant effect on body mass on day 23 of cold exposure (7 d after the beginning of leptin supplementation), by which body time mass had decreased in the 21°C-leptin and 5°C-leptin groups, compared with their counterparts treated with PBS ($F_{1, 12} = 8.45$, $P < 0.05$; fig. 3*A*).

Food Intake. Food intake was not different among the four groups before the experiment (day 0; temperature: $F_{1, 12} = 1.42$, $P > 0.05$; leptin: $F_{1, 12} = 1.03$, $P > 0.05$; post hoc: $P > 0.05$; fig. 3*B*). Cold exposure induced a significant increase in food intake throughout the experiment (day 23; $F_{1, 12} = 41.01$, $P < 0.01$), and intake was significantly higher in the 5°C-PBS and 5°C-leptin groups than in the 21°C-PBS and 21°C-leptin groups (day 23; post hoc: $P < 0.05$). No effect of leptin supplementation on food intake was observed (day 23; $F_{1, 12} = 1.61$, $P > 0.05$; fig. 3*B*).

GEI and Digestibility. Both GEI and DEI were significantly affected by temperature: striped hamsters consumed significantly more food at 5°C than at 21°C (GEI: $F_{1, 10} = 155.56$, $P < 0.001$; post hoc: $P < 0.05$; fig. 4*A*; DEI: $F_{1, 10} = 143.52$, $P < 0.001$; post hoc: $P < 0.05$; fig. 4*B*). Temperature had a significant effect on the GE of feces, which was higher at 5°C than at 21°C ($F_{1, 10} = 117.40$, $P < 0.001$; post hoc: $P < 0.05$; fig. 4*C*). The effect of leptin supplementation on GEI, DEI, and GE of feces was not significant (GEI: $F_{1, 10} = 0.14$, $P > 0.05$; DEI: $F_{1, 10} = 0.15$, $P > 0.05$; GE of feces: $F_{1, 10} = 0.07$, $P > 0.05$). Neither temperature nor leptin supplementation had an effect on di-

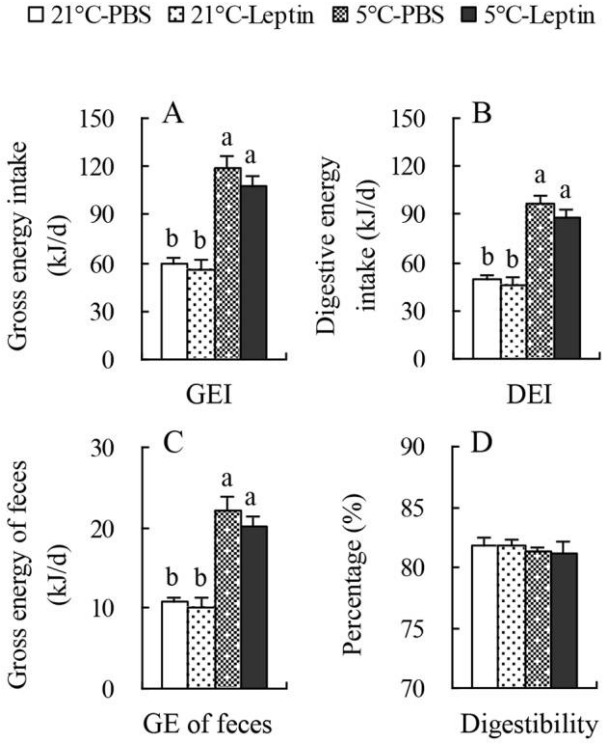

Figure 4. Effect of temperature and leptin supplementation on gross energy intake (GEI; *A*), digestive energy intake (DEI; *B*), gross energy (GE) of feces (*C*), and digestibility (*D*) in striped hamsters. PBS, phosphate-buffered saline.

Table 4: Effect of temperature and leptin supplementation on body composition in striped hamsters

	21°C-PBS	21°C-leptin	5°C-PBS	5°C-leptin	Significance
Body mass (g)	33.5 ± .9	31.1 ± 1.0	34.8 ± 1.4	30.4 ± .6	$P_{Lep} < .05$
Carcass mass (g)	24.3 ± .7	22.6 ± .4	24.6 ± 1.1	21.5 ± .9	$P_{Lep} < .05$
Liver (g)	1.113 ± .067^{B}	1.048 ± .106^{B}	1.637 ± .169^{A}	1.056 ± .073^{B}	$P_{Tem} < .01$
Heart (g)	.148 ± .005	.159 ± .012	.193 ± .019	.185 ± .007	$P_{Tem} < .01$
Lung (g)	.192 ± .011^{B}	.193 ± .011^{B}	.262 ± .011^{A}	.246 ± .014^{A}	$P_{Tem} < .01$
Spleen (g)	.021 ± .002	.027 ± .003	.027 ± .003	.023 ± .004	NS
Kidneys (g)	.334 ± .005^{B}	.372 ± .016^{AB}	.460 ± .033^{A}	.391 ± .021^{AB}	$P_{Tem} < .01$
Stomach (g)	.303 ± .011	.304 ± .021	.372 ± .025	.329 ± .021	$P_{Tem} < .05$
Small intestine (g)	.395 ± .021^{C}	.418 ± .047^{C}	.633 ± .065^{B}	.690 ± .076^{A}	$P_{Tem} < .01$; $P_{Lep} < .05$
Large intestine (g)	.244 ± .019	.180 ± .014	.237 ± .019	.247 ± .014	NS
Cecum (g)	.172 ± .016	.152 ± .030	.188 ± .014	.201 ± .011	NS

Note. PBS, phosphate-buffered saline. Data are means ± SEM. Different letters in the same row indicate significant differences between the treatment groups ($P < 0.05$); P_{Tem} indicates a significant effect of temperature at the level shown; P_{Lep} indicates a significant effect of leptin supplementation at the level shown; NS indicates a nonsignificant difference ($P > 0.05$).

gestibility (temperature: $F_{1, 10} = 0.71$, $P > 0.05$; leptin: $F_{1, 10} = 0.01$, $P > 0.05$; fig. 4*D*).

Body Composition. There were significant effects of temperature on masses of liver, heart, lung, kidneys, stomach, and small intestine, by which cold exposure induced increases in the weight of these organs (table 4). Body mass and carcass mass were affected by leptin supplementation, and leptin supplementation resulted in significant decreases in body mass and carcass mass (7.4% and 7.0%, respectively, at 21°C and 12.8% and 12.6%, respectively, at 5°C; table 4). The mass of the small intestine was affected by leptin supplementation and was significantly higher in the 5°C-leptin group than in the other three groups (table 4).

Body Fat Content and LPL and HL Activity. Body fat content was affected by temperature and was significantly lower at 5°C than at 21°C ($F_{1, 20} = 45.09$, $P < 0.01$; fig. 5*A*). Body fat content was also affected by leptin supplementation, and leptin administration decreased fat content at both 21° and 5°C ($F_{1, 20} = 49.32$, $P < 0.01$; post hoc: $P < 0.05$). Body fat content was positively correlated with the change in body mass ($R^2 = 0.48$, $P < 0.01$). Both LPL and HL activity were affected by leptin supplementation, in that hamsters treated with leptin showed significantly lower LPL and HL activity than hamsters treated with PBS (LPL: $F_{1, 20} = 7.95$, $P < 0.05$; fig. 5*B*; HL: $F_{1, 20} = 22.35$, $P < 0.01$; fig. 5*C*). Temperature had a significant effect on HL activity, and HL activity was significantly decreased at 5°C, compared with that at 21°C ($F_{1, 20} = 8.42$, $P < 0.01$), whereas the effect of cold exposure on LPL activity was not significant ($F_{1, 20} = 1.49$, $P > 0.05$). Body fat content was positively correlated with LPL ($R^2 = 0.43$, $P < 0.01$; fig. 5*D*) and HL activity ($R^2 = 0.72$, $P < 0.01$; fig. 5*E*).

NPY, AgRP, POMC, and CART. NPY and AgRP were not significantly affected by temperature (NPY: $F_{1, 16} = 3.37$, $P = 0.08$; AgRP: $F_{1, 16} = 3.87$, $P = 0.07$) or leptin supplementation (NPY: $F_{1, 16} = 1.80$, $P > 0.05$; fig. 6*A*; AgRP: $F_{1, 16} = 2.18$, $P > 0.05$; fig. 6*B*). No effects of temperature were observed on POMC ($F_{1, 16} = 0.25$, $P > 0.05$; fig. 6*C*), CART ($F_{1, 16} = 1.52$, $P > 0.05$; fig. 6*D*), or Ob-Rb ($F_{1, 16} = 2.93$, $P > 0.05$; fig. 6*E*). There were also no effects of leptin supplementation on POMC ($F_{1, 16} = 0.09$, $P > 0.05$), CART ($F_{1, 16} = 2.42$, $P > 0.05$), or Ob-Rb ($F_{1, 16} = 0.01$, $P > 0.05$).

BAT UCP1 and WAT HSL. BAT UCP1 gene expression was increased in hamsters treated with leptin compared to that in those treated with PBS ($F_{1, 16} = 4.09$, $P = 0.06$; fig. 6*F*). Temperature had a significant effect on BAT UCP1 gene expression,

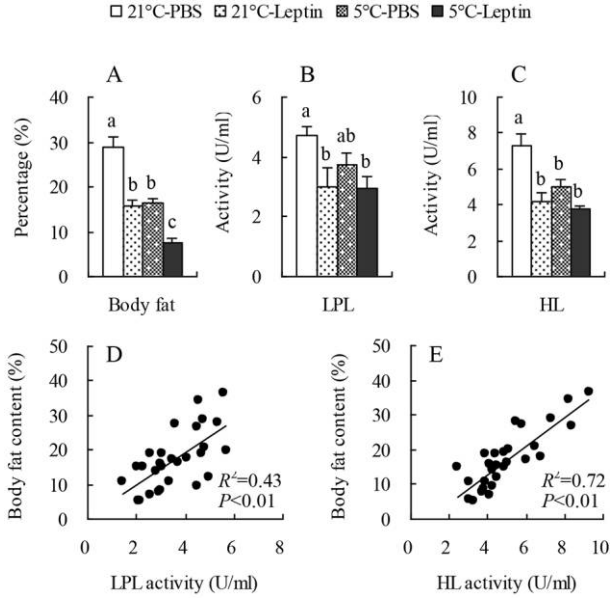

Figure 5. *A–C*, Effect of temperature and leptin supplementation on body fat content (*A*) and lipoprotein lipase (LPL) and hepatic lipase (HL) activity (*B* and *C*, respectively) in striped hamsters. *D*, *E*, Relationships between body fat content and LPL (*D*) and HL (*E*) activity in striped hamsters. PBS, phosphate-buffered saline.

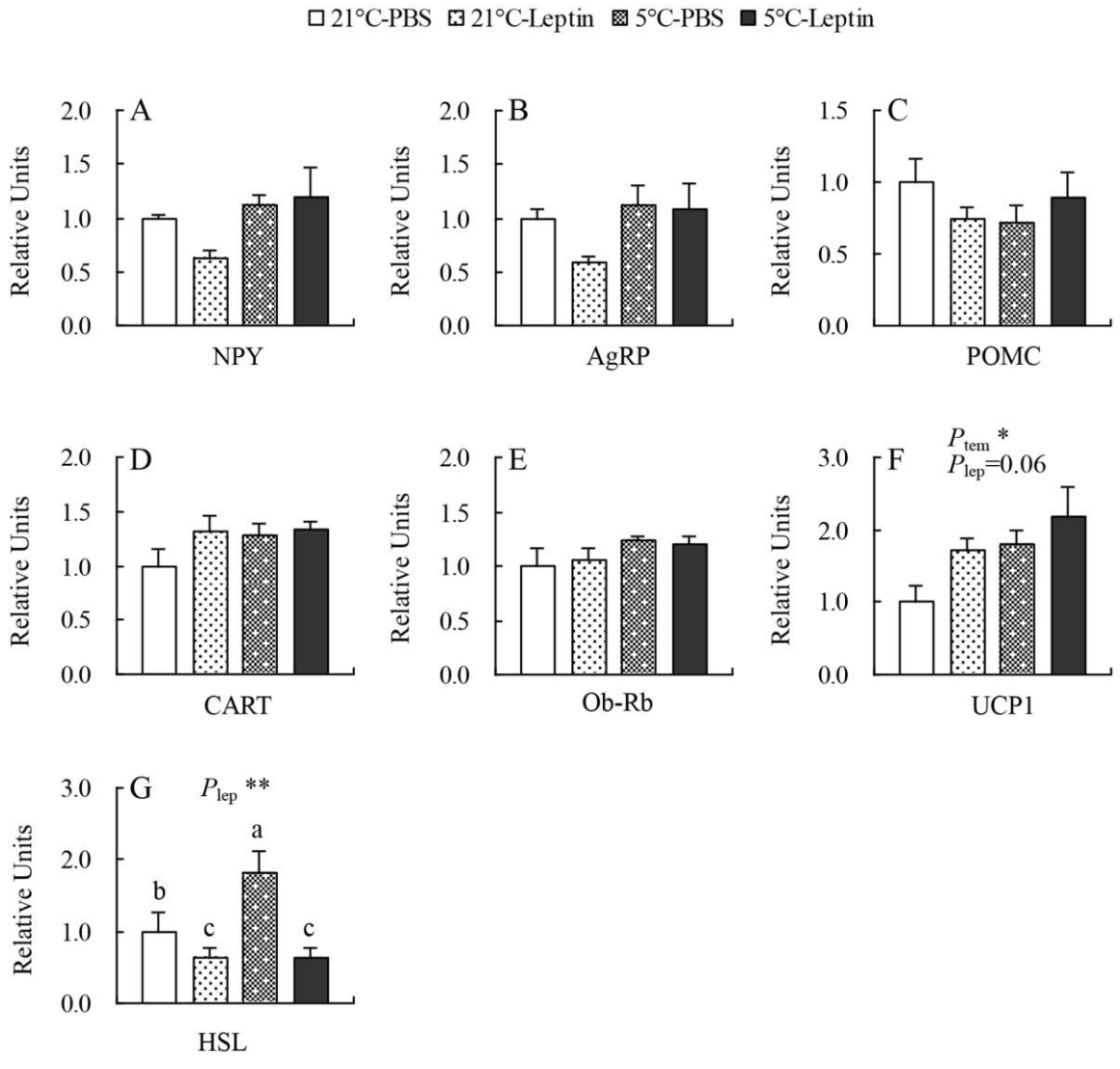

Figure 6. Effects of leptin administration on hypothalamic gene expression of neuropeptide Y (NPY; *A*), agouti-related peptide (AgRP; *B*), proopiomelanocortin (POMC; *C*), cocaine- and amphetamine-regulated transcript (CART; *D*), long-form leptin receptor (Ob-Rb; *E*), brown adipose tissue uncoupling protein 1 (UCP1; *F*), and white adipose tissue hormone-sensitive lipase (HSL; *G*) in striped hamsters. Data are means ± SE. "P_{tem}*" indicates a significant effect of temperature ($P < 0.05$); "P_{lep}**" indicates a significant effect of leptin administration ($P < 0.01$). Different letters in *G* indicate significant differences between the treatment groups ($P < 0.05$). PBS, phosphate-buffered saline.

in that cold exposure increased UCP1 gene expression ($F_{1,16} = 5.14$, $P < 0.05$). WAT HSL was significantly affected by leptin supplementation, and leptin administration led to a significant decrease in WAT HSL gene expression at both 21° and 5°C ($F_{1,16} = 12.33$, $P < 0.01$; post hoc: $P < 0.05$; fig. 6*G*). The effect of temperature on WAT HSL was not significant ($F_{1,16} = 3.34$, $P = 0.08$).

Discussion

This study indicates that striped hamsters showed constant body mass between seasons, which was inconsistent with observations in animals exhibiting seasonal body mass changes, such as golden hamsters (Jefimow et al. 2004), Brandt's voles (Li and Wang 2005*a*), Mongolian gerbils (Li and Wang 2005*b*), and Syrian hamsters (*Mesocricetus auratus*) and Djungarian hamsters (*Phodopus sungorus*; Bartness et al. 2002). The inconsistency might reflect a species-specific response to seasonal changes in environmental factors, especially temperature, photoperiod, and food availability, and might also indicate that different physiological and hormonal mechanisms underpin the regulation of body mass and energy budget. In our study, the energy budget changed significantly over the four seasons. Hamsters in winter consumed much more food and had more energy intake than those in summer. In addition to energy intake, the upper and lower limits to aerobic energy expenditure, as indicated by BMR and NST, also increased in winter

compared with those in summer. The increased energy intake in winter is presumably used mainly to elevate metabolic thermogenesis for thermoregulation, which is a general response to cold winters or winter-like conditions (Mercer 1998; Bartness et al. 2002; Li and Wang 2005*a*, 2005*b*; Wang et al. 2006*a*, 2006*b*).

Unlike body mass, body composition changed significantly in striped hamsters over the four seasons. Hamsters in winter showed heavier inner organs, including liver, lung, and kidney as well as the GI tract, than did those in summer, while body mass was apparently constant; this was likely due to equivalent decreases in the mass of fat. These organs were previously suggested to be active metabolic organs and strongly linked to the rate of metabolism of the whole animal (Daan et al. 1990; Burness et al. 1998; Nespolo et al. 2002; Chi and Wang 2011; Zhao 2011). The increases in the size of the inner organs and the GI tract might be necessary to accommodate the increased energy intake, and consequently they resulted in a higher BMR in winter. Again, these results confirm that animals with a relatively high rate of metabolism had relatively large metabolically active machinery (Hammond and Wunder 1991; Hammond 1993; Konarzewski and Diamond 1995; Speakman and McQueenie 1996; Burness et al. 1998).

As expected, body fat content was the lowest in striped hamsters in winter and the highest in summer. It has been argued that the change in body fat is usually paralleled by serum leptin levels, as has been observed in many small mammals, including laboratory rats and mice (Maffei et al. 1995; Rousseau et al. 2003; Zhao and Wang 2006; Friedman 2011). Unexpectedly, minimum serum leptin levels were found in autumn instead of in winter. This was inconsistent with observations in seasonal mammals that showed the lowest serum leptin in winter, such as Brandt's voles (*Lasiopodomys brandtii*; Li and Wang 2005*a*), Mongolian gerbils (*Meriones unguiculatus*; Li and Wang 2005*b*), root voles (*Microtus oeconomus*; Wang et al. 2006*a*), plateau pikas (*Ochotona curzoniae*; Wang et al. 2006*b*), and Djungarian hamsters (*Phodopus sungorus*; Klingenspor et al. 1996). The reasons for the inconsistency remain unclear, which might reflect that the roles of leptin in striped hamsters are different from those in rodents showing seasonal fluctuations of body mass. It was previously found that leptin had important roles in controlling food intake and increasing energy expenditure in laboratory rodents (Friedman and Halaas 1998; Schwartz et al. 2000; Friedman 2011), whereas in cold-exposed rats leptin gene expression was inhibited, suggesting that the roles of leptin might be associated with ambient temperatures (Puerta et al. 2002). In a number of wild rodents, leptin was found to inhibit energy intake rather than stimulate energy expenditure associated with enhanced metabolic thermogenesis in winter (Li and Wang 2005*a*; Wang et al. 2006*a*, 2006*b*). Thus, a decrease in serum leptin level has been argued to be a starvation signal to increase energy intake in laboratory rats and mice as well as in free-living wild animals (Flier 1998; Li and Wang 2005*b*; Wang et al. 2006*a*, 2006*b*). However, this might not be the case in striped hamsters that were acclimated to seasonal environment and cold conditions. In our study, we did not observe a significant decrease in serum leptin in winter. Leptin administration to striped hamsters had no effect on energy intake at either 21° or 5°C, but it induced a trend of increase in the capacity for thermogenesis, as indicated by upregulation of BAT UCP1 gene expression ($P = 0.06$). We previously observed that serum leptin did not decrease in striped hamsters exposed to a consecutive decease in ambient temperature, in parallel with enhanced thermogenesis (Zhao 2011). It has been also reported that a lack of leptin led to a failure to produce thermogenic reactions to cold stress (Rogers et al. 2009) and that leptin was required for thermogenesis during cold stress (Ukropec et al. 2006). These results suggested that in some animals, such as striped hamsters, leptin might play an important role in increasing maximal thermogenesis in the cold rather than acting as a starvation signal to increase energy intake.

Leptin acts on hypothalamic neuronal targets via interactions with receptors to regulate energy balance, by which leptin engages arcuate hypothalamic neurons expressing putative orexigenic peptides, for example, NPY and AgRP, and anorexigenic peptides, for example, POMC and CART (Pelleymounter et al. 1995; Friedman and Halaas 1998; Ahima et al. 2000; Mercer and Speakman 2001; Rousseau et al. 2003; Friedman 2011). However, in our study leptin administration had no effect on gene expression of Ob-Rb, NPY, AgRP, POMC, and CART in striped hamsters at either 21° or 5°C. This might be the reason for leptin's lack of effect on energy intake. In contrast to the hypothalamic peptides, body fat and LPL and HL activity decreased significantly in hamsters treated with leptin. LPL and HL, expressed in a number of peripheral tissues, including adipose tissue, are key enzymes for hydrolyzing triacylglycerol (TG) circulating in the TG-rich lipoprotein particles in order to deliver fatty acids to the tissue, and the hydrolization of TG was possibly regulated by a number of factors, including leptin (Fielding and Frayn 1998). It was previously reported that leptin treatment significantly lowered LPL activity (Picard et al. 1998). Again, these results indicate that leptin might stimulate lipolysis and consequently attenuate fat accumulation by decreasing LPL and HL activities (Picard et al. 1998; Hikita et al. 2000).

In addition, HSL is classically considered to be the enzyme that catalyzes the rate-limiting step of adipose-tissue lipolysis (Strålfors et al. 1987; Lucas et al. 2003; Fortier et al. 2005). Increases in messenger RNA expression of HSL were found in ob/ob mice treated with leptin for 14 d (Zhang et al. 2008). In our study, we expected that leptin administration to striped hamsters would increase HSL gene expression. However, we unexpectedly observed that HSL gene expression was downregulated in hamsters treated with leptin at either 21° or 5°C. The inconsistency might reflect a response to leptin administration in field animals that is different from that in laboratory rodents, which cannot be easily explained at present. Therefore, further study is likely needed on the relationship between leptin and HSL activity associated with fat mobilization. In addition to the effect of leptin administration, cold exposure increased HSL gene expression by 83% in striped hamsters ($P = 0.08$). However, 0.08 is not quite significant by the criterion of $P < 0.05$. This suggests that HSL gene expression is affected by

ambient temperature, which might be involved in seasonal changes in body fat mass.

Conclusion

Experiments on animals living under natural temperature and photoperiod can provide more realistic and comprehensive information about the role of environmental factors in the regulation of physiology and behavior of the animals. Striped hamsters acclimated to a seasonal environment showed significant increases in food intake and metabolic thermogenesis and heavier inner organs and digestive tracts in winter, compared with those in summer. Body fat content and serum leptin levels also differed over the four seasons. Minimum body fat was observed in winter, whereas minimum serum leptin was found in autumn. This suggests that leptin was perhaps involved in the seasonal regulation of BMR and NST. The role of leptin in striped hamsters, which show a constant body mass over a year, might be different from that in other small mammals that exhibit significant fluctuations of body mass. Cold exposure had significant effects on energy intake, masses of inner organs, body fat content, and serum LPL activity as well as on the gene expression of BAT UCP1 and WAT HSL. Leptin administration to striped hamsters had no effect on energy intake at either 21° or 5°C, but it induced an upregulation of BAT UCP1 gene expression ($P = 0.06$), indicating that leptin plays an important role in increasing maximal thermogenic capacity in cold. These results suggest that in animals like striped hamsters, leptin might play an important role in increasing maximal thermogenesis in the cold rather than acting as a starvation signal to increase energy intake. The gene expression of hypothalamic NPY, AgRP, POMC, and CART were unchanged, whereas body fat and LPL and HL activity were significantly decreased in striped hamsters treated with leptin. This indicates that leptin might stimulate lipolysis and consequently attenuate fat accumulation by decreasing LPL and HL and activities.

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