Hormones and Behavior 110 (2019) 19–28

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

The less weight loss due to modest food restriction drove more fat accumulation in striped hamsters refed with high–fat diet

Jia–Qi Xu, Xiao–Ming Xu, Zhong–Qiang Bi, Lu–Lu Shi, Jing Cao, Zhi–Jun Zhao*

College of Life and Environmental Science, Wenzhou University, Wenzhou 325035, China

ARTICLE INFO

Keywords:
Body fat
Energy intake
Energy metabolism
Food restriction
High–fat diet
Leptin
Refeeding
Striped hamsters

ABSTRACT

Food restriction (FR) has been commonly used to decrease body fat, reducing the risk of overweight in humans and animals. However, the lost weight has been shown to be followed by overweight when food restriction ends. It remains uncertain whether the weight loss drives the overweight, or not. In the present study, striped hamsters were restricted by 15%, 30% and 40% of *ad libitum* food intake for 2 weeks, followed by high–fat refeeding for 6 weeks (FR15%–Re, FR30%–Re and FR40%–Re). The hamsters in FR15%, FR30% and FR40% groups decreased by 21.1%, 37.8% and 50.0% in fat mass ($P < 0.01$), and 16.8%, 42.8% and 53.4% in leptin levels ($P < 0.01$) compared with the hamsters fed *ad libitum*. The FR15%–Re, FR30%–Re and FR40%–Re groups showed 77.0%, 37.2% and 23.7% more body fat than *ad libitum* group ($P < 0.01$). The FR15%–Re group showed considerable decreases in gene expression of arcuate nucleus co–expressing proopiomelanocortin (POMC), cocaine – and amphetamineregulated transcript (CART) and the long isoform of leptin receptor (LepRb) in the hypothalamus and of several genes associated with fatty acid transport to mitochondria and β–oxidation in brown adipose tissue and liver. It suggests that less weight loss is likely to drive more fat accumulation when food restriction ends, in which the impaired function of LepRb, POMC and CART in the brain and fatty acid oxidation in brown adipose tissue and liver may be involved.

1. Introduction

Food restriction has been shown to lengthen life span in a variety of animals (Osborne et al., 1917; McCay et al., 1935; Weindruch et al., 1986; Kirkwood and Shanley, 2005) and to decrease body mass in laboratory rodents and humans (Weigle et al., 1997; Speakman and Mitchell, 2011; Gotthardt and Bello, 2017). The majority of the weight lost during food restriction is accounted for by loss of body fat (Harris and Martin, 1984; Cleary et al., 1987; Mitchell et al., 2015). It has been reported that under food restriction both fat mass and visceral fat are reduced to approximately one–third of that of *ad libitum* fed animals, while lean body mass is unchanged (Barzilai et al., 1998; Speakman and Mitchell, 2011). Given the global problem of obesity and associated comorbidities in humans, food restriction has been therefore commonly used to decrease body fat, reducing the risk of obesity (Cameron and Speakman, 2011; Haslam and James, 2005). However, periods of restricted food intake that lead to lowered body mass have been shown to be followed by rapid regain of the lost weight after reintroduction of *ad libitum* feeding (Cleary, 1986; Vink et al., 2016; Gotthardt and Bello, 2017). The regain of body weight has been observed to lead to overweight in a number of species and under certain conditions, that is, a considerable increase in body fat during refeeding period compared to original fat mass (Szepesi and Epstein, 1977; Bosy-Westphal et al., 2013; Simonds et al., 2018).

It is well established that the changes in body mass and fat are largely affected by the trade–off between energy intake and energy expenditure (Zhang et al., 2012). A number of studies in rodents and humans suggest that the enhanced energy efficiency after a period of food restriction possibly leads to positive energy balance, consequently resulting in over–weight and obesity (Szepesi and Epstein, 1977). Moreover, sustained restriction is associated with neuroendocrine changes promoting hunger, leading to hyperphagia (Hambly et al., 2007), thus making mass regain even more likely (Cameron and Speakman, 2011). Both physiological and behavioral strategies in response to food restriction have been extensively studied, however, the roles of experience of an organism under food restriction in the regain of body weight and development of obesity following *ad libitum* refeeding have received less attention (Speakman and Mitchell, 2011).

Leptin is a mainly adipocyte–secreted protein, and production and circulating levels of leptin are highly correlated with fat deposition (Zhang et al., 1994; Friedman and Halaas, 1998; Leibel, 2002; Sucajtys-Szulc et al., 2009; Zhao et al., 2014a). It has been proposed that leptin

* Corresponding author at: College of Life and Environmental Science, Wenzhou University, Wenzhou, Zhejiang 325035, China.
E-mail address: zhaozj@wzu.edu.cn (Z. Zhao).

https://doi.org/10.1016/j.yhbeh.2019.02.010
Received 2 November 2018; Received in revised form 7 February 2019; Accepted 13 February 2019
Available online 23 February 2019
0018-506X/

signals reflecting nutritional state are sensed by the hypothalamus, which, in turn, modulates food intake and energy expenditure (Hetherington and Ranson, 1942; Kennedy, 1953; Halaas et al., 1995; Friedman and Halaas, 1998; Ahima and Osei, 2004; Park and Ahima, 2014). Leptin mediates its effects by binding to the long isoform of leptin receptor (LepRb) expressed in the hypothalamus and other areas of the brain (Park and Ahima, 2014). Leptin directly targets two neuronal populations in the arcuate nucleus co–expressing proopiomelanocortin (POMC)/cocaine– and amphetamineregulated transcript (CART), and agouti–related peptide (AgRP) and neuropeptide Y (NPY) (Xu et al., 2011). Leptin stimulates POMC/CART expression and inhibits AgRP/NPY expression, thereby reducing food intake, increasing energy expenditure, and decreasing body weight. Fat depots are considerably mobilized by an organism to cope with restricted intake, resulting in decreased leptin levels, *i.e.* hypoleptinemia (Rosenbaum and Leibel, 2014). Hypoleptinemia following and during weight loss suggests that the major physiological function of leptin is to signal states of negative energy balance and decreased energy stores (Rosenbaum and Leibel, 2014). The negative status of energy balance indicated by hypoleptinemia may increase the drive to eat, which likely leads to hyperphagia and favors weight regain when food restriction ends. It may be expected that the subjects under severe food restriction will develop lower leptin levels than those under moderate restriction, providing stronger signals to the brain, resulting in more fat accumulation and even obesity.

In addition to the brain, peripheral organs, such as liver, skeletal muscle, and adipose tissue can integrate the expenditure and nutritional, neuronal, and hormonal signals to ensure metabolic homeostasis, contributing to the fine regulation of body mass (Auffret et al., 2012). Different from white adipose tissue (WAT), brown adipose tissue (BAT) contains several lipid droplets and numerous mitochondria, where a unique protein, uncoupling protein 1 (UCP1) is expressed and distributed, that supports the thermogenic function of brown adipocytes (Cannon and Nedergaard, 2004; Ricquier, 2005). The brown adipocyte burns lipids to produce heat, making large amounts of fatty acids available quickly for mitochondrial uncoupling and consequently thermogenesis (Ricquier, 2005). Carnitine palmitoyltransferase–1 (CPT–1) is the main regulator of long–chain fatty acids transport across the mitochondrial membrane for beta–oxidation (McGarry and Brown, 1997; Park and Cook, 1998; Foster, 2004; Song et al., 2004). CPT–1α is the primary isoform expressed in the liver while the CPT–1β is highly expressed in BAT and skeletal muscle. CPT–1β is stimulated *via* direct interactions of the peroxisome proliferator activated receptor α (PPARα) and the peroxisome proliferator activated receptor gamma coactivator (PGC–1α) (Vega et al., 2000; Song et al., 2004). PPARα is a nuclear transcription factor that induces the expression of genes involved in fatty acid transport to mitochondria to undergo β–oxidation (Evans et al., 2004; Patsouris et al., 2006; Song et al., 2010). PGC–1α was initially identified in BAT, and is highly expressed in tissues with high metabolic rates including BAT, muscle and liver (Puigserver et al., 1998; Puigserver and Spiegelman, 2003).

The striped hamster (*Cricetulus barabensis*) is a major rodent in northern China and is also distributed in Russia, Mongolia, and Korea (Shi et al., 2017). The hamsters feed on foraging crop seeds in winter, but do not store food and feed on stems and leaves of plant during summer (Tan et al., 2017). We have previously observed that striped hamsters showed physiological and behavioral strategies to cope with the period of food shortage, such as food restriction and deprivation (Zhao and Cao, 2009; Zhao et al., 2014a, 2014b; Wen et al., 2018; Zhao et al., 2018). Food–restricted hamsters also show considerable decreases of fat depots and serum leptin levels (Zhao, 2012; Zhao et al., 2013; Zhao et al., 2014a). Inconsistent with other animals including laboratory mice and rats, the food–restricted hamsters showed weight regain when restriction ended, but did not develop overweight or obesity (Zhao et al., 2013; Zhao et al., 2014a, 2014b). However, it is unclear if hypoleptinemia is involved in the resistance to overweight, or not, during *ab libitum* refeeding period. Since leptin repletion is most effective following weight loss by food restriction (Hambly et al., 2012), in the present study striped hamsters were subjected to food restriction treatment at different levels, *i.e.* by 15%, 30% and 40% of *ad libitum* food intake, followed by refeeding of a high–fat diet. It hypothesized that the restricted animals with a gradient of leptin levels to the brain would have different phenotype associated with weight regain, overweight or obesity when food restriction ended. We predicted that the lower leptin levels to the brain during food restriction would drive more fat accumulation when food restriction ended, resulting in overweight and obesity.

2. Materials and methods

2.1. Animals

Eighty one male striped hamsters, with 3.5–4.5 months of age, were used in this study. The striped hamsters showed considerable seasonal fluctuations of body fat (Zhao et al., 2010; Zhao et al., 2014c) and therefore is suitable for addressing the scientific objectives of this study. All protocols and procedures were approved by the Animal Care and Use Committee at the Wenzhou University in accordance with the Chinese Council on Animal Care guidelines and conformed to ARRIVE guidelines for reporting animal research. These hamsters were obtained from our laboratory breeding colony at Wenzhou University, which started with the animals that were initially trapped from farmland at the center of Hebei province (115°C13′E, 38°C12′S), North China Plain. Animals were housed individually in plastic cages (29 cm × 18 cm × 16 cm) with sawdust bedding, and kept at 21 ± 1 °C under a 12 L:12D (light:dark, lights on at 08:00 am) photoperiod. Food (standard rodent chow, 17.6 kJ/g; Beijing KeAo Feed Company, Beijing, China) and tap water were provided *ad libitum*. The animals were randomly divided into one of four groups: one Ad group (n = 18), hamsters were fed with normal diet (standard rodent chow) throughout the experiment, and three food restriction and refeeding (FR–Re) groups, *i.e.* the animals were restricted by 15%, 30% and 40% of *ad libitum* food intake for 2 weeks and refed *ad libitum* with high–fat diet (fat was 60%, carbohydrate was 20% and protein was 20%, calorific value was 22.0 kJ/g, Research Diet, D12492, USA) for 6 weeks, briefly as FR15%–Re (n = 22), FR30%–Re (n = 22) and FR40%–Re (n = 19), thereafter.

2.2. Body mass and food intake

At the onset of the experiment, animals were weighed on a daily basis to the nearest 0.1 g to establish baseline body mass for one week. The *ad libitum* food intake was also measured daily during the baseline measurements. Food intake was calculated from the difference between the initial food provided and the uneaten food on the next day, subtracting food residues mixed in the bedding materials. The amount of food provided during the period of food restriction treatment in FR15%–Re, FR30%–Re and FR40%–Re groups were calculated from the averaged *ad libitum* food intake during the baseline measurements.

2.3. Energy intake and digestibility

Gross energy intake (GEI) and digestibility were measured over the last two days of food restriction and of refeeding, using a food balance method described previously (Grodzinski and Wunder, 1975; Zhao et al., 2018). A known quantity of food was provided, and 48 h later, any uneaten food and orts mixed with the bedding material were collected, along with feces from each animal. Food and feces were separated manually after drying at 60 °C to constant mass. The gross energy contents of the food and feces were determined using an IKA C2000 oxygen bomb calorimeter (IKA, Germany). GEI, gross energy of feces (GEF), digestive energy intake (DEI) and digestibility were calculated as

the following equation:

GEI (kJ/d) = [food provided (g/d)
× dry matter content of food (%)
–dry spillage of food and uneaten food]
× gross energy content of food (kJ/g); GEF (kJ/d)
= dry feces mass (g/d) × gross energy content of feces (kJ/g); DEI (kJ/d) = GEI–GEF; and digestibility (%)
= DEI/GEI × 100%

2.4. Resting metabolic rate (RMR) and daily metabolic rate (DMR)

The RMR and DMR were measured in thirty two randomly selected hamsters form the Ad and three food–restricted groups at the end of food restriction, and also in forty nine hamsters from the four groups after the 6 weeks of refeeding. Both RMR and DMR were determined using an O_2 module high–speed sensor unit (994620–CSHSP–01) for calorimetric measurements in an open–flow respirometry system (TSE, Germany). As described previously (Wen et al., 2018; Xu et al., 2018), air was pumped through a cylindrical sealed Perspex chamber at a rate of 1 L/min. Gases leaving the chamber were dried and sampled using an oxygen analyzer at a flow rate of 0.38 L/min. Data were collected every 10 s by a computer connected *via* an analogue–to–digital converter (TSE, Germany). Animals were transferred into the chambers without food for 1 h for the adaptation purpose, and were measured for further 3 h at 30 ± 0.5 °C, within the thermal neutral zone of this hamster species (Zhao et al., 2010; Zhao et al., 2014d). The consecutive minimum rates of oxygen consumption over 10 min were taken to calculate RMR.

The DMR was determined over 24 h at 21 ± 0.5 °C (the same temperature at which hamsters were kept), during which the animals under food restriction were provided with restricted food, and the animals during refeeding were provided with food *ad libitum*. Tap water was provided *ad libitum* for all the animals during the measurements. The DMR was calculated from the consecutive rat of oxygen consumption. Both RMR and DMR were corrected to standard temperature and air pressure conditions, and expressed as mLO_2/h.

2.5. Serum leptin

On the day next to the DMR measurements, animals were sacrificed and trunk blood was collected and allowed to clot for 3 h at 4 °C. The blood was then centrifuged at 4 °C, 3500 *g* for 10 min. Serum was taken and stored at −80 °C until assayed. Leptin concentrations were determined in food–restricted subjects, using a radio–immunoassay kit (Linco Research Inc., St. Charles, MO), following the standard kit instructions. As described previously (Zhao et al., 2013), the commercial kit was valid for the striped hamster, and the lower and upper limits of the assay kit were 1 and 50 ng/mL. The inter– and intra–assay variations were 3.6% and 8.7%, respectively.

2.6. Body fat

After blood had been collected, the hypothalamus, BAT, subcutaneous fat, and liver were separated carefully and quickly. All the tissues were frozen in liquid nitrogen and stored at −80 °C until analysis. Then the stomach, small and large intestine, caecum and liver, heart, lung, spleen and kidneys were removed. The remaining carcass was weighed to determine wet mass, dried to a constant mass in an oven at 60 °C for two weeks, 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, and the weight difference before and after extraction was taken to calculate fat mass and fat content of carcass.

Table 1
Gene-specific primer sequences used for Real-time RT-qPCR analysis.

Gene	Primers (5′ to 3′)	Size (bp)
Actin (forward)	AAAGACCTCTATGCCAACA	196
Actin (reverse)	ACATCTGCTGGAAGGTGG	
AgRP (forward)	TGTTCCCAGAGTTCCCAGGTC	227
AgRP (reverse)	ATTGAAGAAGCGGCAGTAGCAC	
CART (forward)	TACCTTTGCTGGGTGCCG	260
CART (reverse)	AAGTTCCTCGGGGACAGT	
CPT1α (forward)	TCGACAGGTGGTTTGACAAGTCC	247
CPT1α (reverse)	TCTATGACCTCCTGGCATTCTCC	
CPT1β (forward)	TGGTGGGCAACTAACTATG	186
CPT1β (reverse)	GTCCAGTTTGCGGCGATA	
CPT2 (forward)	CAGGGCTTTGACCGACAC	127
CPT2 (reverse)	AGTGCTGGTGGACAGGATGT	
LepR (forward)	CAGTGTCGATACAGCTTGGA	200
LepR (reverse)	TTGCATATTTAACTGAGGGT	
Leptin (forward)	AACCCTCATCAAGACCATT	240
Leptin (reverse)	GCCAGCAGATGGAGAAGG	
NPY (forward)	ACCCTCGCTCTGTCCCTG	186
NPY (reverse)	AATCAGTGTCTCAGGGCTA	
PGC1α (forward)	GAACAAGACTATTGAGCGAACC	204
PGC1α (reverse)	GAGTGGCTGCCTTGGGTA	
POMC (forward)	GGTGGGCAAGAAGCGACG	205
POMC (reverse)	CTTGTCCTTGGGCGGGCT	
PPARα (forward)	CAGCCCCTCATCAGTCAG	121
PPARα (reverse)	GACTCCGTAGTGGTAGCCT	
UCP1 (forward)	GGGACCATCACCACCCTGGCAAAAA	330
UCP1 (reverse)	GGCTTTCTGTTGTGGCTAT	

AgRP, agouti-related protein; CART; cocaine- and amphetamine-regulated transcript.; CPT1α, carnitine palmitoyltransferase 1 alpha; CPT1β, carnitine palmitoyltransferase 1 beta; CPT2, carnitine palmitoyltransferase 2; LepR, long form of the leptin receptor; NPY, neuropeptide Y; PGC1α, peroxisome proliferator activated receptor gamma coactivator 1 alpha; POMC, pro-opiomelanocortin; PPARα, peroxisome proliferator activated receptor alpha; UCP1, uncoupling protein 1.

2.7. Real–time RT–qPCR analysis

Total RNA of subcutaneous fat, hypothalamus, BAT and liver were extracted using TRIzol Reagent (TAKARA, Dalian, China). The cDNA was synthesized in a final reaction volume of 50 μL with random primer oligo (dT)18 and AMV Reverse Transcriptase (TAKARA). 2 μL cDNA samples were taken for the subsequent PCR reaction using gene–specific primers (Table 1). The final reaction volume of 20 μL contained 10 μL of 2 × SYBR Premix EX Tag TM (TAKARA), 0.4 μL of forward prime and reverse primer (final concentration 0.2 μM per primer), 2 μL cDNA template and 7.2 μL DEPC H_2O. qPCR was performed using Roche Light Cycler480II real–time qPCR system. Actin was used as an internal standard. Samples were quantified for relative quantity of gene expression (Zhao et al., 2014a).

2.8. Statistical analysis

Data were analyzed using SPSS 21.0 statistic software. Before statistical analysis, the data were confirmed by Kolmogorov–Smirnov and Levene tests to coincide with the normal distribution and variance homogeneity. The effects of food restriction and refeeding on body mass and food intake on any day points were examined using a one–way ANOVA, followed by Tukey *post hoc* tests where required. The difference in body mass and food intake over the course of the experiment was examined using repeated–measures ANOVA. The effects of food restriction and refeeding on RMR and DMR were examined one–way ANCOVA, with body mass as a covariate. The energy intake, digestibility and fat content, as well as gene expression were analyzed also using one–way ANOVA, followed by Tukey *post hoc* tests where required. Eta-squared (η^2) was used to measure effect size in ANOVA. Pearson's correlation was performed to examine relationships between body mass lost and regain and body fat content. All data were

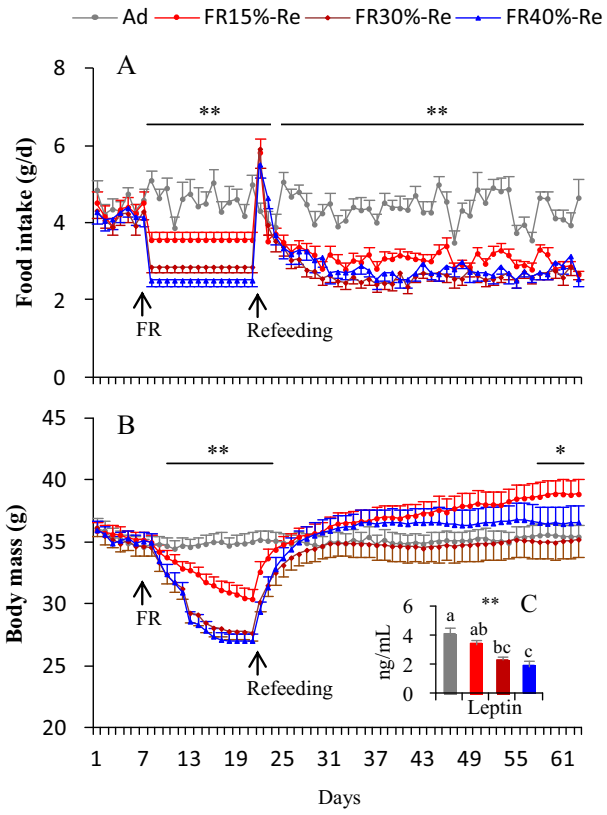

Fig. 1. A: food intake and B: body mass of the food-restricted striped hamsters refed with high-fat diet, and C: leptin concentration of food-restricted hamsters. Ad, the animals were fed *ad libitum* throughout the experiment; FR15%-Re, FR30%-Re, FR40%-Re, the animals were restricted by 15%, 30% and 40% of *ad libitum* food intake, respectively, for 2 weeks and followed by high-fat diet refeeding for 6 weeks. Data are means ± s.e.m. *, the significant effects of food restriction or refeeding ($P < 0.05$), **, $P < 0.01$. Different letters above the columns indicate significant difference between the groups ($P < 0.05$).

means ± s.e.m. Statistical significance was assumed at $P < 0.05$.

3. Results

3.1. Food intake

The four groups did not differ in food intake during the period of baseline measurement (day 1, $F_{3,45} = 0.96$, $P > 0.05$, $\eta^2 = 0.060$; day 7, $F_{3,45} = 0.61$, $P > 0.05$, $\eta^2 = 0.039$, Fig. 1A). During the period of food restriction (day 8 to 21), the hamsters in FR15%, FR30% and FR40% groups consumed significantly less food than the hamsters fed *ad libitum* (day 8, $F_{3,45} = 30.26$, $P < 0.01$, $\eta^2 = 0.668$; day 21, $F_{3,45} = 28.93$, $P < 0.01$, $\eta^2 = 0.659$). The food intake increased significantly when food restriction ended, and considerably fell back one day later in FR15%–Re, FR30%–Re and FR40%–Re groups compared to that of *ad libitum* group (day 22, $F_{3,45} = 3.38$, $P < 0.05$, $\eta^2 = 0.184$). There were significant differences among the four groups almost throughout the refeeding period, during which FR15%–Re, FR30%–Re and FR40%–Re groups showed significantly lower food intake than Ad group (day 25, $F_{3,45} = 10.95$, $P < 0.01$, $\eta^2 = 0.422$, day 63, $F_{3,45} = 15.94$, $P < 0.01$, $\eta^2 = 0.515$, Fig. 1A).

3.2. Body mass

Body mass did not differ among the four groups during the period of baseline measurement (day 1, $F_{3,45} = 0.03$, $P > 0.05$, $\eta^2 = 0.001$; day 7, $F_{3,45} = 0.15$, $P > 0.05$, $\eta^2 = 0.009$, Fig. 1B). Body mass of the hamsters considerably decreased following the food restriction on day 8, and it decreased by 13.6%, 20.3% and 22.9%, respectively, in the FR15%, FR30% and FR40% groups on day 21 compared to that on day 7 (repeated measures ANOVA, days 7–21, FR15%, $F_{14,182} = 30.72$, $P < 0.01$, $\eta^2 = 0.703$; FR30%, $F_{14,182} = 145.37$, $P < 0.01$, $\eta^2 = 0.917$; FR40%, $F_{14,140} = 151.27$, $P < 0.01$, $\eta^2 = 0.937$), while it did not change significantly in the hamsters fed *ad libitum* (repeated measures ANOVA, days 7–21, ad, $F_{14,182} = 1.56$, $P > 0.05$, $\eta^2 = 0.147$). Food restriction had significant effects on body mass on day 10 and thereafter (day 10, $F_{3,45} = 5.76$, $P < 0.01$, $\eta^2 = 0.284$), and FR15%, FR30% and FR40% groups were lower by 13.2%, 21.1% and 22.8% respectively, than Ad groups (day 21, $F_{3,45} = 34.21$, $P < 0.01$, $\eta^2 = 0.616$, *post hoc*, $P < 0.05$, Figs. 1B, 2A). Body mass regained significantly when food restriction ended, and it increased by 28.9%, 27.6% and 35.3%, respectively, in the FR15%–Re, FR30%–Re and FR40%–Re groups on day 64 relative to that on day 21 (repeated

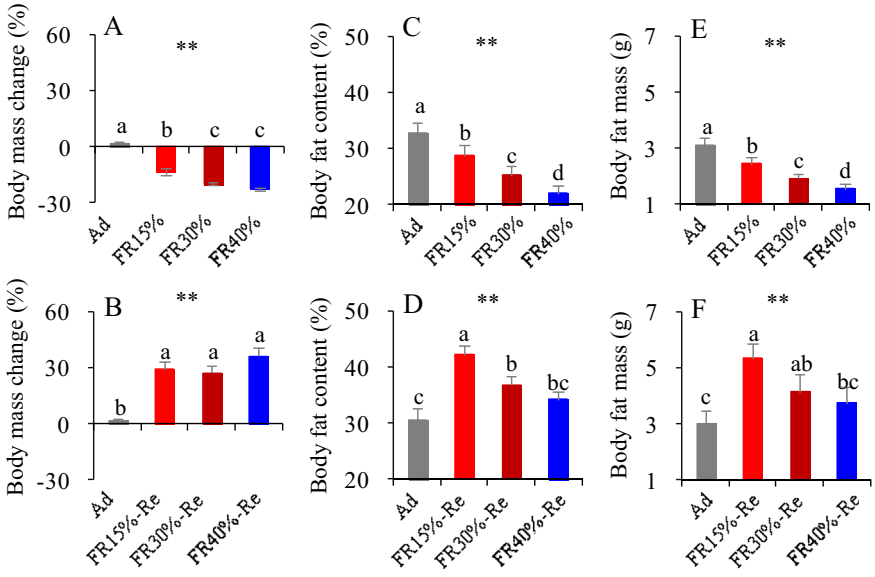

Fig. 2. Body mass change (A, B), body fat content (C, D) and body fat mass (E, F) of the food-restricted striped hamsters refed with high-fat diet. Ad, the animals were fed *ad libitum* throughout the experiment; FR15%, FR30% and FR40%, the animals were restricted by 15%, 30% and 40% of *ad libitum* food intake for 2 weeks, and followed by high-fat diet refeeding for 6 weeks (−Re). Data are means ± s.e.m. *, $P < 0.05$, the significant effects of food restriction (A, C, E) or refeeding (B, D, F); **, $P < 0.01$. Different letters above the columns indicate significant difference between the groups ($P < 0.05$).

measures ANOVA, days 21–64, FR15%–Re, $F_{43,559} = 27.79$, $P < 0.01$, $\eta^2 = 0.655$; FR30%–Re, $F_{43,559} = 26.00$, $P < 0.01$, $\eta^2 = 0.666$; FR40%–Re, $F_{43,430} = 29.20$, $P < 0.01$, $\eta^2 = 0.744$, Figs. 1B, 2B), while it did not change significantly in the hamsters fed *ad libitum* (repeated measures ANOVA, days 21–64, Ad, $F_{14,387} = 0.97$, $P > 0.05$, $\eta^2 = 0.009$). The significant effects of refeeding on body mass were observed on day 59 and thereafter (day 59, $F_{3,45} = 2.92$, $P < 0.05$, $\eta^2 = 0.168$). At the end of refeeding, the group difference in body mass was significant (day 63, $F_{3,45} = 2.78$, $P < 0.05$, $\eta^2 = 0.161$), by which the body mass of FR15%–Re group was higher by 10.7% than that of Ad group, whereas it did not significantly differ between FR30%–Re, FR40%–Re groups and Ad group (*post hoc*, $P > 0.05$, Fig. 1B).

3.3. Serum leptin

Serum leptin concentrations were significantly affected by food restriction, and the hamsters in FR15%, FR30% and FR40% groups decreased by 16.8%, 42.8% and 53.4% in leptin levels compared with the hamsters fed *ad libitum* ($F_{3,28} = 7.88$, $P < 0.01$, $\eta^2 = 0.457$, *post hoc*, $P < 0.05$, Fig. 1C). Additionally, the FR40% group showed 44.0% lower leptin levels than FR15% group (*post hoc*, $P < 0.05$).

3.4. Body fat

Body fat content was significantly affected by food restriction, and it averaged 32.6% in the hamsters fed *ad libitum*, and was decreased considerably in the food–restricted hamsters ($F_{3,28} = 7.31$, $P < 0.01$, $\eta^2 = 0.439$, Fig. 2C). Body fat content was decreased to 28.6% in FR15% group and reduced to 21.9% in FR40% group, indicating that fat mobilization was greater in the hamsters subjected to sever restriction (*post hoc*, $P < 0.05$). Body fat content was also significantly different among the Ad and FR15%–Re, FR30%–Re and FR40%–Re groups ($F_{3,45} = 3.97$, $P < 0.05$, $\eta^2 = 0.217$, Fig. 2D). Interestingly, the maximum fat content was observed in FR15%–Re, rather than in FR40% group (*post hoc*, $P < 0.05$). Consistent with fat content, body fat mass decreased by 21.1%, 37.8% and 50.0%, respectively, in FR15%, FR30% and FR40% groups compared with that in Ad group ($F_{3,28} = 9.06$, $P < 0.01$, $\eta^2 = 0.492$, Fig. 2E). The refeeding resulted in a considerable increase in fat mass, which increased by 77.0%, 37.2% and 23.7% in the FR15%–Re, FR30%–Re and FR40%–Re groups relative to Ad group ($F_{3,45} = 2.87$, $P < 0.05$, $\eta^2 = 0.166$, *post hoc*, $P < 0.05$, Fig. 2F).

The percentage of body mass lost was positively correlated with body mass regain in FR15%–Re ($r = 0.72$, $P < 0.01$) and FR30%–Re groups ($r = 0.64$, $P < 0.05$), while no significant correlations were observed in Ad ($r = -0.31$, $P > 0.05$) or FR30%–Re group ($r = 0.02$, $P > 0.05$, Fig. 3A). There was a negative correlation between body mass lost and body fat content in Ad group ($r = -0.71$, $P < 0.05$), and no significant correlations were observed in FR15%–Re ($r = 0.01$, $P > 0.05$), FR30%–Re ($r = 0.32$, $P > 0.05$) or FR40%–Re group ($r = 0.03$, $P > 0.05$, Fig. 3B).

3.5. Energy intake and digestibility

The hamsters subjected to the FR15%, FR30% and FR40% treatment showed 9.3%, 19.7 and 32.9% lower GEI, and produced 7.4%, 16.1% and 32.8% less feces than the hamsters fed *ad libitum* (GEI, $F_{3,38} = 12.78$, $P < 0.01$, $\eta^2 = 0.577$, Fig. 4A; GEF, $F_{3,38} = 7.81$, $P < 0.01$, $\eta^2 = 0.455$, Fig. 4B). The DEI was also significantly affected by food restriction, and it was significantly lower in the food–restricted groups than that of Ad group (DEI, $F_{3,38} = 10.20$, $P < 0.01$, $\eta^2 = 0.522$, Fig. 4C). The digestibility was not affected by food restriction, and that of three food–restricted groups was similar to that of Ad groups ($F_{3,38} = 0.09$, $P > 0.05$, $\eta^2 = 0.009$, Fig. 4D). At the end of the refeeding period, the hamsters in FR15%–Re, FR30%–Re and FR40%–Re groups had lower GEI and GEF than the animals fed *ad libitum* (GEI, $F_{3,45} = 11.35$, $P < 0.01$, $\eta^2 = 0.431$, Fig. 4E; GEF, $F_{3,45} = 180.01$, $P < 0.01$, $\eta^2 = 0.923$, Fig. 4F). The hamsters in three FR–Re groups showed 14.1% lower DEI, but 13.5% higher digestibility, on average, than Ad group (DEI, $F_{3,45} = 3.31$, $P < 0.05$, $\eta^2 = 0.181$, Fig. 4G; Digestibility, $F_{3,45} = 105.55$, $P < 0.01$, $\eta^2 = 0.875$, Fig. 4H).

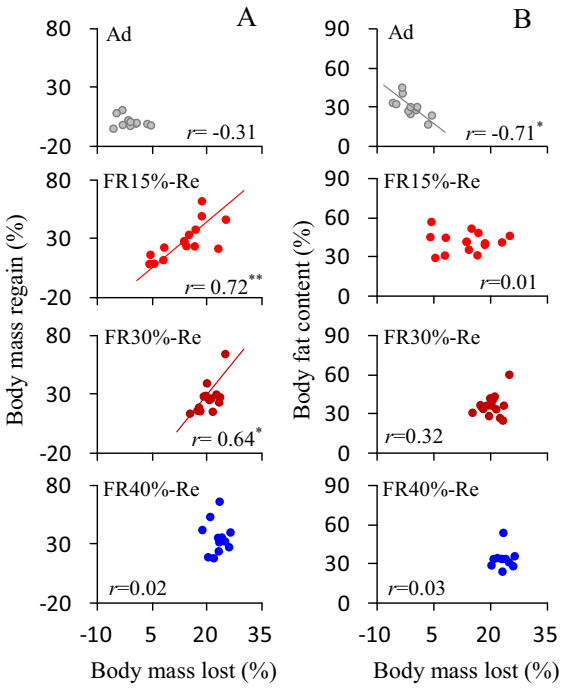

Fig. 3. Correlations between body mass lost during 2 weeks of food restriction and body mass regain (A) and body fat content (B) of striped hamsters refed with high-fat diet for 6 weeks. Ad, animals were fed *ad libitum* throughout the experiment; FR15%-Re, FR30%-Re, FR40%-Re, animals were restricted by 15%, 30% and 40% of *ad libitum* food intake, respectively, for 2 weeks and followed by high-fat diet refeeding for 6 weeks. *, significant correlation ($P < 0.05$), **, $P < 0.01$.

3.6. Resting metabolic rate (RMR) and daily metabolic rate (DMR)

RMR was significantly affected by food restriction, and it deceased by 24.7%, 25.6%, and 23.9%, respectively, in the FR15%, FR30% and FR40% groups compared with Ad group ($F_{3,27} = 7.95$, $P < 0.01$, $\eta^2 = 0.427$, Fig. 5A). At the end of refeeding period, RMR was significantly different among the four groups, and it was higher by 10.0%, 12.2% and 23.6% in FR15%–Re, FR30%–Re and FR40%–Re groups compared with Ad group ($F_{3,44} = 3.75$, $P < 0.05$, $\eta^2 = 0.260$, Fig. 5B).

The DMR of Ad hamsters and food–restricted hamsters showed considerable fluctuations over 24 h, and it decreased during the light phase and increased during the dark phase (supplementary materials, Fig. S1A, B, C). Interestingly, the DMR tended to be lower in the three restricted groups during daytime relative to that in Ad group, whereas the DMR during nighttime was not different between the Ad and food–restricted groups. The DMR of FR15%–Re, FR30%–Re and FR40%–Re also showed considerable fluctuations over 24 h, consistent with that observed in Ad group. No notable differences among the four groups were observed during the light or dark phases (supplementary materials, Fig. S1D, E, F).

3.7. Gene expression of neuropeptides in the brain and of leptin in subcutaneous fat

There was no significant difference in NPY or AgRp gene expression

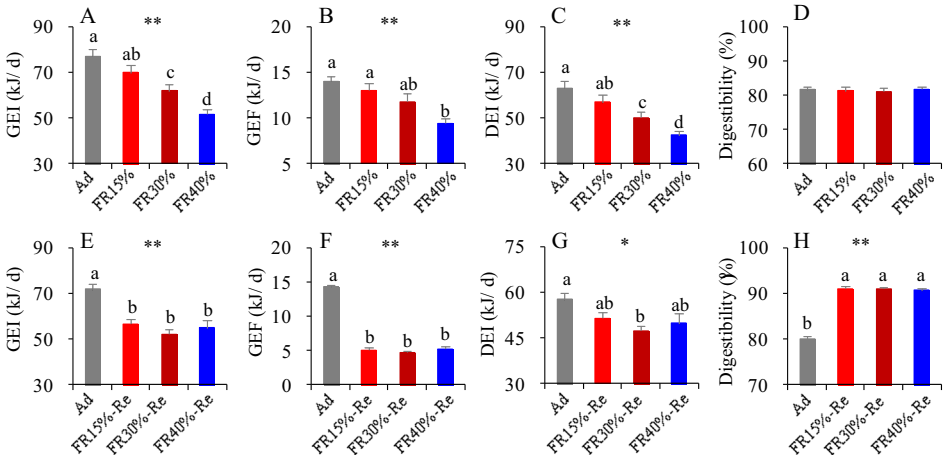

Fig. 4. Gross energy intake (GEI, A, E), gross energy of feces (GEF, B, F), digestive energy intake (DEI, C, G) and digestibility (D, H) of the food-restricted striped hamsters refed with high-fat diet. Ad, the animals were fed *ad libitum* throughout the experiment; FR15%, FR30% and FR40%, the animals were restricted by 15%, 30% and 40% of *ad libitum* food intake for 2 weeks, and followed by high-fat diet refeeding for 6 weeks (−Re). Data are means ± s.e.m. *, $P < 0.05$, the significant effects of food restriction (A, B, C, D) or refeeding (E, F, G, H), **, $P < 0.01$. Different letters above the columns indicate significant difference between the groups ($P < 0.05$).

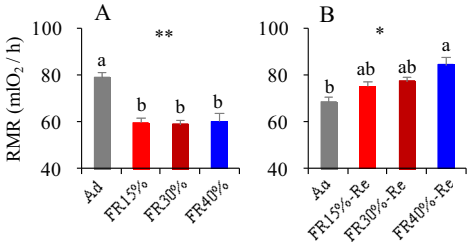

Fig. 5. Resting metabolic rate (RMR) of the food-restricted striped hamsters refed with high-fat diet. Ad, the animals were fed *ad libitum* throughout the experiment; FR15%, FR30% and FR40%, the animals were restricted by 15%, 30% and 40% of *ad libitum* food intake for 2 weeks, and followed by high-fat diet refeeding for 6 weeks (−Re). Data are means ± s.e.m. *, $P < 0.05$, the significant effects of food restriction (A) or refeeding (B), **, $P < 0.01$. Different letters above the columns indicate significant difference between the groups ($P < 0.05$).

in the hypothalamus among the four groups (NPY, $F_{3,\ 45} = 2.06$, $P > 0.05$, $\eta^2 = 0.068$, Fig. 6A; AgRp, $F_{3,\ 45} = 1.42$, $P > 0.05$, $\eta^2 = 0.048$, Fig. 6B). The hypothalamus POMC gene expression was significantly different among the four groups, and it was down–regulated by 61.1% and 59.0% in the FR15%–Re group compared with that in Ad and FR40%–Re groups, respectively ($F_{3,\ 45} = 4.99$, $P < 0.01$, $\eta^2 = 0.151$, *post hoc*, $P < 0.05$, Fig. 6C). CART gene expression also differed significantly among the groups, and the lower gene expression was observed in the FR15%–Re group ($F_{3,\ 45} = 7.07$, $P < 0.01$, $\eta^2 = 0.201$, *post hoc*, $P < 0.05$, Fig. 6D). The gene expression of LepR in the hypothalamus was considerably different among the four groups, and the minimum was observed in the FR15%–Re group, with decreasing by 85.4% and 85.8%, respectively, relative to that in Ad and FR40%–Re groups ($F_{3,\ 45} = 6.22$, $P < 0.01$, $\eta^2 = 0.181$, *post hoc*, $P < 0.05$, Fig. 6E). Contrary to that observed in LepR, leptin gene expression in subcutaneous fat was considerably up–regulated in the FR15%–Re group, with increasing by 5.1 and 3.5–fold relative to that in Ad and FR40%–Re groups, respectively ($F_{3,\ 45} = 17.06$, $P < 0.01$, $\eta^2 = 0.381$, *post hoc*, $P < 0.05$, Fig. 6F).

3.8. Gene expression of PPARα, PGC1α, CPT1β and UCP1 in BAT

The PPARα gene expression in BAT differed significantly among the four groups, and it was down–regulated by 60.9% in FR15%–Re group compared with that of FR40%–Re group ($F_{3,\ 45} = 4.25$, $P < 0.01$, $\eta^2 = 0.113$, *post hoc*, $P < 0.05$, Fig. 7A). Consistently, the minimum gene expression of PGC1α was observed in FR15%–Re group, that was down–regulated by 73.4% relative to that of FR40%–Re group ($F_{3,\ 45} = 4.38$, $P < 0.01$, $\eta^2 = 0.116$, *post hoc*, $P < 0.05$, Fig. 7B). The group difference was also observed in CPT1β gene expression, which tended to decrease in the FR15%–Re and FR30%–Re groups ($F_{3,\ 45} = 3.56$, $P < 0.05$, $\eta^2 = 0.096$, Fig. 7C). The UCP1 gene expression tended to be up–regulated in FR40%–Re group, which increased by 55.8% and 46.4%, respectively, compared to that of Ad and FR15%–Re groups, whereas the group difference was not statistically significant ($F_{3,\ 45} = 2.11$, $P > 0.05$, $\eta^2 = 0.059$, Fig. 7D).

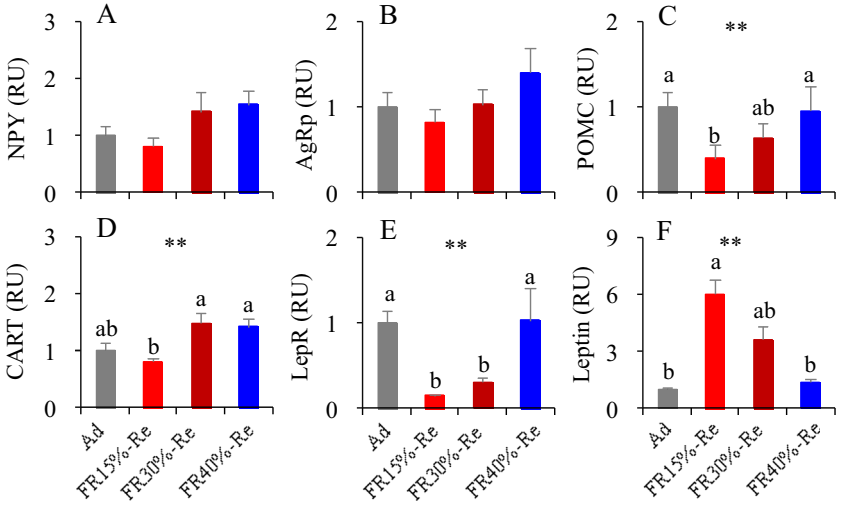

Fig. 6. Gene expression of NPY (A), AgRp (B), POMC (C), CART (D) and LepR (E) in the hypothalamus and of leptin (F) in subcutaneous fat of the food-restricted striped hamsters refed with high-fat diet. Ad, the animals were fed *ad libitum* throughout the experiment; FR15%-Re, FR30%-Re, FR40%-Re, the animals were restricted by 15%, 30% and 40% of *ad libitum* food intake, respectively, for 2 weeks and followed by high-fat diet refeeding for 6 weeks. Data are means ± s.e.m. *, the significant effects of refeeding ($P < 0.05$), **, $P < 0.01$. Different letters above the columns indicate significant difference between the groups ($P < 0.05$).

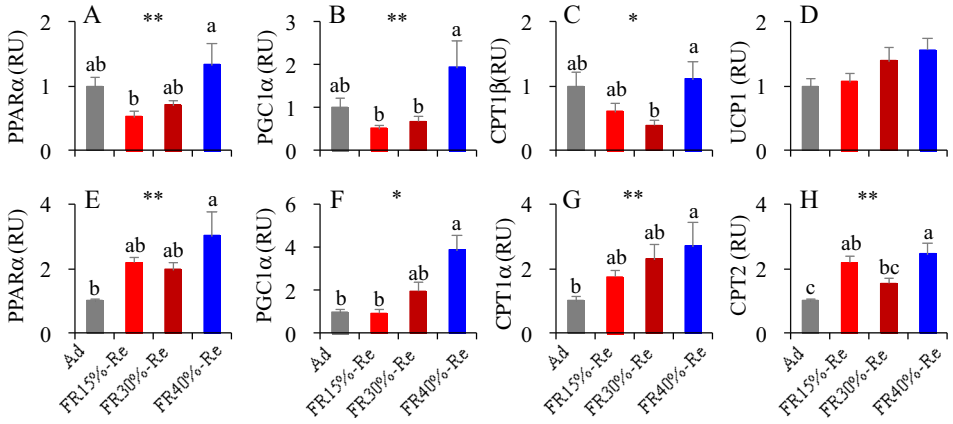

Fig. 7. Gene expression of PPARα (A), PGC1α (B), CPT1β (C) and UCP1 (D) in BAT and of PPARα (E), PGC1α (F), CPT1α (G) and CPT2 (H) in liver of the food-restricted striped hamsters refed with high-fat diet. Ad, the animals were fed *ad libitum* throughout the experiment; FR15%-Re, FR30%-Re, FR40%-Re, the animals were restricted by 15%, 30% and 40% of *ad libitum* food intake, respectively, for 2 weeks and followed by high-fat diet refeeding for 6 weeks. Data are means ± s.e.m. *, the significant effects of refeeding ($P < 0.05$), **, $P < 0.01$. Different letters above the columns indicate significant difference between the two groups ($P < 0.05$).

3.9. Gene expression of PPARα, PGC1α, CPT1α and CPT2 in liver

There were significant group differences in PPARα gene expression in liver, which was up–regulated in FR40%–Re group relative to that in Ad group ($F_{3,\ 45} = 4.62$, $P < 0.01$, $\eta^2 = 0.123$, *post hoc*, $P < 0.05$, Fig. 7E). The PGC1α gene expression differed significantly among the four groups, and it was up–regulated by about 3–fold in FR40%–Re group compared with that of Ad and FR15%–Re group ($F_{3,\ 45} = 3.71$, $P < 0.05$, $\eta^2 = 0.107$, *post hoc*, $P < 0.05$, Fig. 7F). Consistently, the maximum levels of CPT1α gene expression observed in FR40%–Re group, with increasing by 1.7–fold relative to Ad group ($F_{3,\ 45} = 2.65$, $P < 0.05$, $\eta^2 = 0.075$, *post hoc*, $P < 0.05$), and by 55.4% compared to FR15%–Re group (*post hoc*, $P > 0.05$, Fig. 7G). In addition, the CPT2 gene expression was significantly up–regulated in the FR15%–Re and FR40%–Re groups relative to that in Ad group ($F_{3,\ 45} = 8.01$, $P < 0.05$, $\eta^2 = 0.195$, *post hoc*, $P < 0.05$, Fig. 7H).

4. Discussion

The striped hamsters decreased body mass and fat content during food restriction and regained lost weight considerably, exhibiting similar patterns to that in the animals reported previously (Szepesi and Epstein, 1977; Cleary, 1986; Bosy-Westphal et al., 2013; Zhao et al., 2014a; Vink et al., 2016; Gotthardt and Bello, 2017; Simonds et al., 2018). The hamsters under severe food restriction (restricted by 40% of *ad libitum* food intake) showed lower fat content and leptin levels compared with those subjected to moderate food restriction (restricted by 15% of *ad libitum* food intake). Unexpectedly, the subjects with lower leptin levels seemed not to develop over–weight and obesity during high–fat diet refeeding compared with that their counterparts with higher leptin levels.

The food–restricted animals and humans are generally characterized by weight loss (Cleary, 1986; Zhao et al., 2014a, 2014b; Vink et al., 2016). In the present study, food-restricted striped hamsters considerably decreased their body mass compared with the hamsters fed *ad libitum*. It has been previously reported that the enhanced mobilization of fat depots mainly contributes the lost weight during food restriction (Harris and Martin, 1984; Cleary et al., 1987; Barzilai et al., 1998; Speakman and Mitchell, 2011; Mitchell et al., 2015). Consistently, we observed a significant decrease in body fat content in food restricted hamsters. The hamsters under severe restriction (FR40%) showed considerably lower body fat compared to those subjected to moderate food restriction (FR15%), suggesting that the animals had to increase fat mobilization to cope with severe food shortage.

When restriction ended, the striped hamsters showed a considerable regain of lost weight, as described in other animals and the same strain of hamsters (Cleary, 1986; Vink et al., 2016; Gotthardt and Bello, 2017; Zhao et al., 2018). In the present study, body mass of food–restricted hamsters recovered to the control levels within 4 days of *ad libitum* refeeding. The food–restricted laboratory rats lost weight and recovered after 6 days of refeeding (Harris and Martin, 1984). Laboratory mice were also observed to regain their weight soon after *ad libitum* refeeding (Cameron and Speakman, 2011). The regain of lost weight was mainly due to replacement of fat depots, indicating that animals with the experience of food shortage were able to increase energy storage when food was plentiful, and consequently increased the capacity to cope with unexpected food shortage in the future (Zhao et al., 2013; Speakman, 2018). Interestingly, in the present study the modest food-restricted hamsters regained more weight after 6 weeks of high-fat refeeding and accumulated considerably more fat relative to the severe restriction groups. These findings indicated that the magnitudes of weight regain and fat accumulation might be dependent on the extent to which animals were restricted. It is known that adipose tissue and plasma leptin concentrations are dependent on the amount of energy stored as fat, as well as the status of energy balance, therefore, the extent to which fat is stored is an indicator of changes in energy balance (Ahima and Flier, 2000; Zhao et al., 2014b; Wen et al., 2018). The strong negative energy balance in an animal with minimum fat storage under severe food restriction might not lead to overweight and obesity during refeeding, whereas the weak negative energy balance under moderate restriction likely did.

Lost weight in food–restricted animals was usually caused by limited food intake (Cleary et al., 1987; Hambly et al., 2007). Here, the food-restricted hamsters consumed less diet and showed considerably lower DEI compared to the hamsters fed *ad libitum*. Neither food–restricted groups nor *ad libitum* group showed significantly differences in digestibility. It suggested that the food–restricted hamsters failed to enhance the energy efficiency to compensate for the limited food provided. When restriction ended, hyperphagia occurred in animals refed *ad libitum* (Szepesi and Epstein, 1977; Cleary, 1986; Hambly et al., 2007). Inconsistently, in the present study the food–restricted hamsters increased food intake only one day after refeeding of high–fat diet, and thereafter consumed less food than Ad hamsters. Digestibility was significantly increased in the hamsters refed with high–fat diet compared with that of controls, which possibly contributed body weight regain and fat accumulation.

It has been observed that food–restricted animals had less body fat and lower leptin, which would predispose to rebound hyperphagia, resulting in regain of lost weight (Woods et al., 1998; Ogawa et al., 2005). Leptin supplementation to food–restricted animals reduced food intake and prevented regaining of the lost body mass (Fernández-Galaz et al., 2002). This indicated that in restricted animals the hunger signal of lower leptin levels seemed more effective during refeeding than the satiety signals arising from the gastrointestinal tract (Widdowson et al., 1997; Ogawa et al., 2005). As mentioned above, we hypothesized that the animals under severe food restriction would have considerable lower leptin production than those with moderate restriction, which might be sensed by the brain, leading to more considerable

hyperphagia. In the present study, striped hamsters did show a significant down–regulation of serum leptin levels, consistent with that observed in many other animals (Ahima et al., 1996; Hambly et al., 2012; Sucajtys-Szulc et al., 2009; Zhao et al., 2014a). In detail, leptin levels were decreased by 16.8%, 42.8% and 53.4% in FR15%, FR30% and FR40% groups compared with that of controls. Circulating leptin levels are in proportion to body fat mass, thus serving as an adiposity signal of the total body energy stores (Frederich et al., 1995; Maffei et al., 1995). Either absolute (genetic) or relative (restricted intake) leptin deficiency, or the both, lead to increases in hunger (Leibel, 2002). In the current study, the hamsters under severe food restriction showed lower leptin levels, and therefore might be more hunger than those subjected to modest food restriction. Unexpected, overweight and obesity occurred in the FR15%–Re group following high–fat diet refeeding, rather than in the FR30%–Re and FR40%–Re groups, suggesting that in food–restricted subjects a moderate decrease of leptin might be more effective to drive weight regain and obesity during refeeding.

It is well known that leptin, binding to specific LepRb in the hypothalamus, inhibits orexigenic peptides AgRP/NPY expression and stimulates anorexigenic peptides POMC/CART expression, thereby reducing food intake (Zhang et al., 1994; Elmquist et al., 1998; Schwartz et al., 1996; Scott et al., 2009; Gautron and Elmquist, 2011; Xu et al., 2011; Park and Ahima, 2014). In the present study, both WAT leptin expression and serum leptin levels in refeeding groups rebounded to the control levels in parallel with the fat mass regain, suggesting that the leptin might act differently in refed animals from that in food–restricted subjects. Neither NPY nor AgRp differed in the three FR–Re groups from that in Ad group. The POMC and CART were significantly down–-regulated in FR85%–Re group compared to FR60%–Re group or Ad group. The possible explanation was that leptin signal transmission during high–fat refeeding might be effective in animals with the experience of severe restriction, whereas it might be impaired in the animals under moderate restriction. The explanation was supported by the fact that LepRb expression in FR85%–Re group was down–regulated by 85.4% compared with that in Ad group, and by 85.8% compared with FR60%–Re group. As described previously, leptin controls energy balance and body weight primarily by targeting LepRb –expressing neurons in the brain, particularly in the hypothalamus (Zhang et al., 1994; Zhou and Rui, 2013). Either leptin deficiency or defects in the components of the leptin signaling pathways causes obesity (Zhou and Rui, 2013). In mice, lack of functional leptin receptor (LepRb) displays the obesity phenotypes (Tartaglia et al., 1995; Bates and Myers Jr, 2003; Myers et al., 2008). Restoration of LEPRb expression attenuates the obesity phenotypes of LepRb–deficient animals (Morton et al., 2003; Zhou and Rui, 2013). Leptin acts *via* LepRb to stimulate the synthesis of anorexigenic peptides POMC and CART, is required for leptin to promote weight loss (Fan et al., 1997; Schwartz et al., 1997; Cowley et al., 2001; Zhou and Rui, 2013). Many genetic variants of the POMC have been identified to be associated with development of obesity (Krude et al., 1998). The finding from the present study indicated that leptin failed to inhibit overweight and obesity in the hamsters under moderate FR and high–fat diet refeeding might partly due to the impaired function of POMC and CART.

In the present study, gene expression of BAT PPARα, PGC–1α and CPT–1β was significantly up–regulated in FR40%–Re group compared to that in FR15%–Re group. BAT UCP1 gene expression was also significantly higher in FR40%–Re group than FR15%–Re group. It suggested that BAT might increase the transportation of fatty acids and consequently elevated the rate of beta–oxidation and mitochondrial uncoupling in the animal under severe food shortage relative to moderate food restriction. Consistently, the hamsters in FR40%–Re group also showed significant up–regulation of PPARα, PGC–1α and CPT–1β gene expression compared to the subjects in FR15%–Re group. It has been found that the oxidation of long chain fatty acids in the liver is elevated with high fat diets, in parallel with the increased expression of the PPARα, PGC–1α and CPT–1α gene (Park et al., 1995; Howell 3rd. et al., 2009; Cook et al., 2001; Sugden et al., 2002). PPARα plays a significant role in maintaining fatty acid β–oxidation during refeeding, and in PPARα deficient mice mitochondrial fatty acid oxidation is not stimulated (Kersten et al., 1999; Sugden et al., 2002; Ramakrishnan et al., 2016). Additionally, over–expression of PGC–1α induced the expression of CPT–1α in the liver, suggesting that PGC–1α stimulates hepatic fatty acid oxidation (Song et al., 2004; Zhang et al., 2004; Ma et al., 2005). In the present study, the PPARα, PGC–1α and CPT–1α gene expression was considerably down–regulated in the FR15%–Re group relative to that in FR40%–Re group, suggesting that fatty acid oxidation might be impaired in the animals under the experience of moderate food shortage, whereas it might be more effective for the animals experiencing severe food restriction.

5. Conclusions

The striped hamsters under severe food restriction decreased more body mass and fat, and showed lower leptin levels compared with their counterparts subjected to moderate food restriction. When food restriction ended, the lost weight regained considerably. Unexpectedly, the hamsters with lower leptin levels regained less weight and had significantly less fat accumulation than those under moderate food restriction. The POMC and CART gene expression in the hypothalamus was significantly down–regulated in the hamsters under moderate food restriction, in paralleled with a considerable down–regulation of LepRb expression, compared with that in the subjects with severe food restriction. It suggested that leptin signal transmission during high–fat refeeding might be effective in animals with the experience of severe restriction, whereas it might be impaired in the animals with moderate restriction. Additionally, there were considerably down–regulations of BAT PPARα, PGC–1α and CPT–1β, and of liver PPARα, PGC–1α and CPT–1α in the hamsters with the experience of moderate food restriction, suggesting that fatty acid oxidation may be impaired, consequently contributing to the development of overweight.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2019.02.010.

Acknowledgements

This work was supported by grants (No. 31670417; 31870388) from the National Natural Science Foundation of China.

References

Ahima, R.S., Flier, J.S., 2000. Adipose tissue as an endocrine organ. Trends Endocrinol. Metab. 11, 327–332.
Ahima, R.S., Osei, S.Y., 2004. Leptin signaling. Physiol. Behav. 81, 223–241.
Ahima, R.S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E., Flier, J.S., 1996. Role of leptin in the neuroendocrine response to fasting. Nature 382, 250–252.
Auffret, J., Viengchareun, S., Carré, N., Denis, R.G., Magnan, C., Marie, P.Y., Muscat, A., Fève, B., Lombès, M., Binart, N., 2012. Beige differentiation of adipose depots in mice lacking prolactin receptor protects against high-fat-diet-induced obesity. FASEB J. 26, 3728–3737.
Barzilai, N., Banerjee, S., Hawkins, M., Chen, W., Rossetti, L., 1998. Caloric restriction reverses hepatic insulin resistance in aging rats by decreasing visceral fat. J. Clin. Invest. 101, 1353–1361.
Bates, S.H., Myers Jr., M.G., 2003. The role of leptin receptor signaling in feeding and neuroendocrine function. Trends Endocrinol. Metab. 14, 447–452.
Bosy-Westphal, A., Schautz, B., Lagerpusch, M., Pourhassan, M., Braun, W., Goele, K., Heller, M., Glüer, C.C., Müller, M.J., 2013. Effect of weight loss and regain on adipose tissue distribution, composition of lean mass and resting energy expenditure in young overweight and obese adults. Int. J. Obes. 37, 1371–1377.
Cameron, K.M., Speakman, J.R., 2011. Reduction of dietary energy density reduces body mass regain following energy restriction in female mice. J. Nutr. 141, 182–188.
Cannon, B., Nedergaard, J., 2004. Brown adipose tissue: function and physiological significance. Physiol. Rev. 84, 277–359.
Cleary, M.P., 1986. Consequences of restricted feeding/refeeding cycles in lean and obese female Zucker rats. J. Nutr. 116, 290–303.
Cleary, M.P., Muller, S., Lanza-Jacoby, S., 1987. Effects of long-term moderate food restriction on growth, serum factors, lipogenic enzymes and adipocyte glucose

metabolism in lean and obese Zucker rats. J. Nutr. 117, 355–360.
Cook, G.A., Edwards, T.L., Jansen, M.S., Bahouth, S.W., Wilcox, H.G., Park, E.A., 2001. Differential regulation of carnitine palmitoyltransferase-I gene isoforms (CPT-I alpha and CPT-I beta) in the rat heart. J. Mol. Cell. Cardiol. 33, 317–329.
Cowley, M.A., Smart, J.L., Rubinstein, M., Cerdan, M.G., Diano, S., Horvath, T.L., Cone, R.D., Low, M.J., 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 411, 480–484.
Elmquist, J.K., Bjorbaek, C., Ahima, R.S., Flier, J.S., Saper, C.B., 1998. Distributions of leptin receptor mRNA isoforms in the rat brain. J. Comp. Neurol. 395, 535–547.
Evans, R.M., Barish, G.D., Wang, Y.X., 2004. PPARs and the complex journey to obesity. Nat. Med. 10, 355–361.
Fan, W., Boston, B.A., Kesterson, R.A., Hruby, V.J., Cone, R.D., 1997. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385, 165–168.
Fernández-Galaz, C., Fernández-Agulló, T., Pérez, C., Peralta, S., Arribas, C., Andrés, A., Carrascosa, J.M., Ros, M., 2002. Long-term food restriction prevents ageing-associated central leptin resistance in wistar rats. Diabetologia 45, 997–1003.
Foster, D.W., 2004. The role of the carnitine system in human metabolism. Ann. N. Y. Acad. Sci. 1033, 1–16.
Frederich, R.C., Hamann, A., Anderson, S., Lollmann, B., Lowell, B.B., Flier, J.S., 1995. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat. Med. 1, 1311–1314.
Friedman, J.M., Halaas, J.L., 1998. Leptin and the regulation of body weight in mammals. Nature 395, 763–770.
Gautron, L., Elmquist, J.K., 2011. Sixteen years and counting: an update on leptin in energy balance. J. Clin. Invest. 121, 2087–2093.
Gotthardt, J.D., Bello, N.T., 2017. Meal pattern alterations associated with intermittent fasting for weight loss are normalized after high-fat diet re-feeding. Physiol. Behav. 174, 49–56.
Grodzinski, W., Wunder, B.A., 1975. Ecological energetics of small mammals. In: Golley, F.B., Petrusewicz, K., Ryszkowski, L. (Eds.), Small Mammals: Their Productivity and Population Dynamics. Cambridge Univ. Press, Cambridge, pp. 173–204.
Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., Friedman, J.M., 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269, 543–546.
Hambly, C., Mercer, J.G., Speakman, J.R., 2007. Hunger does not diminish over time in mice under protracted caloric restriction. Rejuvenation Res. 10, 33–41.
Hambly, C., Duncan, J.S., Archer, Z.A., Moar, K.M., Mercer, J.G., Speakman, J.R., 2012. Repletion of TNFα or leptin in calorically restricted mice suppresses post-restriction hyperphagia. Dis. Model. Mech. 5, 83–94.
Harris, R.B., Martin, R.J., 1984. Recovery of body weight from below "set point" in mature female rats. J. Nutr. 114, 1143–1150.
Haslam, D.W., James, W.P.T., 2005. Obes. Lancet 366, 1197–1209.
Hetherington, A.W., Ranson, S.W., 1942. The spontaneous activity and food intake of rats with hypothalamic lesions. Am. J. Phys. 136, 609–617.
Howell 3rd., G., Deng, X., Yellaturu, C., Park, E.A., Wilcox, H.G., Raghow, R., Elam, M.B., 2009. N-3 polyunsaturated fatty acids suppress insulin-induced SREBP-1c transcription via reduced trans-activating capacity of LXRalpha. Biochim. Biophys. Acta 1791, 1190–1196.
Kennedy, G.C., 1953. The role of depot fat in the hypothalamic control of food intake in the rat. Proc. R. Soc. Lond. B 140, 578–592.
Kersten, S., Seydoux, J., Peters, J.M., Gonzalez, F.J., Desvergne, B., Wahli, W., 1999. Peroxisome proliferatoractivated receptor alpha mediates the adaptive response to fasting. J. Clin. Invest. 103, 1489–1498.
Kirkwood, T.B.L., Shanley, D.P., 2005. Food restriction, evolution and ageing. Mech. Ageing Dev. 126, 1011–1016.
Krude, H., Biebermann, H., Luck, W., Horn, R., Brabant, G., Gruters, A., 1998. Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nat. Genet. 19, 155–157.
Leibel, R.L., 2002. The role of leptin in the control of body weight. Nutr. Rev. 60, S15–S19.
Ma, K., Zhang, Y., Elam, M.B., Cook, G.A., Park, E.A., 2005. Cloning of the rat pyruvate dehydrogenase kinase 4 gene promoter: activation of pyruvate dehydrogenase kinase by the peroxisome proliferatoractivated receptor {gamma} coactivator. J. Biol. Chem. 280, 29525–29532.
Maffei, M., Halaas, J., Ravussin, E., Pratley, R.E., Lee, G.H., Zhang, Y., Fei, H., Kim, S., Lallone, R., Ranganathan, S., 1995. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med. 1, 1155–1161.
McCay, C., Crowell, M., Maynard, L., 1935. The effect of retarded growth upon the length of the life span and upon the ultimate body size. J. Nutr. 10, 63–79.
McGarry, J.D., Brown, N.F., 1997. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur. J. Biochem. 244, 1–14.
Mitchell, S.E., Tang, Z., Kerbois, C., Delville, C., Konstantopedos, P., Bruel, A., Derous, D., Green, C., Aspden, R.M., Goodyear, S.R., Chen, L., Han, J.J., Wang, Y., Promislow, D.E., Lusseau, D., Douglas, A., Speakman, J.R., 2015. The effects of graded levels of calorie restriction: I. Impact of short term calorie and proteinrestriction on body composition in the C57BL/6 mouse. Oncotarget 6, 15902–15930.
Morton, G.J., Niswender, K.D., Rhodes, C.J., Myers Jr., M.G., Blevins, J.E., Baskin, D.G., Schwartz, M.W., 2003. Arcuate nucleus-specific leptin receptor gene therapy attenuates the obesity phenotype of Koletsky rats. Endocrinology 144, 2016–2024.
Myers, M.G., Cowley, M.A., Munzberg, H., 2008. Mechanisms of leptin action and leptin resistance. Annu. Rev. Physiol. 70, 537–556.
Ogawa, R., Strader, A.D., Clegg, D.J., Sakai, R.R., Seeley, R.J., Woods, S.C., 2005. Chronic food restriction and reduced dietary fat: risk factors for bouts of overeating. Physiol. Behav. 86, 578–585.
Osborne, T.B., Mendel, L.B., Ferry, E.L., 1917. The effect of retardation of growth upon the breeding period and duration of life in rats. Science 45, 294–295.
Park, H.K., Ahima, R.S., 2014. Leptin Signaling. F1000Prime Rep. 6. pp. 73.
Park, E.A., Cook, G.A., 1998. Differential regulation in the heart of mitochondrial carnitine palmitoyltransferase-I muscle and liver isoforms. Mol. Cell. Biochem. 180, 27–32.
Park, E.A., Mynatt, R.L., Cook, G.A., Kashfi, K., 1995. Insulin regulates enzyme activity, malonyl-CoA sensitivity and mRNA abundance of hepatic carnitine palmitoyltransferase-I. Biochem. J. 310, 853–858.
Patsouris, D., Reddy, J.K., Müller, M., Kersten, S., 2006. Peroxisome proliferator-activated receptor α mediates the effects of high-fat diet on hepatic gene expression. Endocrinology 147, 1508–1516.
Puigserver, P., Spiegelman, B.M., 2003. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. Endocr. Rev. 24, 78–90.
Puigserver, P., Wu, Z., Park, C.W., Graves, R., Wright, M., Spiegelman, B.M., 1998. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92, 829–839.
Ramakrishnan, S.K., Khuder, S.S., Al-Share, Q.Y., Russo, L., Abdallah, S.L., Patel, P.R., Heinrich, G., Muturi, H.T., Mopidevi, B.R., Oyarce, A.M., Shah, Y.M., Sanchez, E.R., Najjar, S.M., 2016. PPARα (peroxisome proliferator-activated receptor α) activation reduces hepatic CEACAM1 protein expression to regulate fatty acid oxidation during fasting-refeeding transition. J. Biol. Chem. 291, 8121–8129.
Ricquier, D., 2005. Respiration uncoupling and metabolism in the control of energy expenditure. Proc. Nutr. Soc. 64, 47–52.
Rosenbaum, M., Leibel, R.L., 2014. 20 years of leptin: role of leptin in energy homeostasis in humans. J. Endocrinol. 223, 83–96.
Schwartz, M.W., Seeley, R.J., Campfield, L.A., Burn, P., Baskin, D.G., 1996. Identification of targets of leptin action in rat hypothalamus. J. Clin. Invest. 98, 1101–1106.
Schwartz, M.W., Seeley, R.J., Woods, S.C., Weigle, D.S., Campfield, L.A., Burn, P., Baskin, D.G., 1997. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 46, 2119–2123.
Scott, M.M., Lachey, J.L., Sternson, S.M., Lee, C.E., Elias, C.F., Friedman, J.M., Elmquist, J.K., 2009. Leptin targets in the mouse brain. J. Comp. Neurol. 514, 518–532.
Shi, L.L., Tan, S., Wen, J., Zhao, Z.J., 2017. Individual differences in BMR and energetic strategies of striped hamsters in response to a high fat diet. Acta Theriol. Sin. 37, 179–188.
Simonds, S.E., Pryor, J.T., Cowley, M.A., 2018. Repeated weight cycling in obese mice causes increased appetite and glucose intolerance. Physiol. Behav. 26, 184–190.
Song, S., Zhang, Y., Ma, K., Jackson-Hayes, L., Lavrentyev, E.N., Cook, G.A., Elam, M.B., Park, E.A., 2004. Peroxisomal proliferator activated receptor gamma coactivator (PGC-1alpha) stimulates carnitine palmitoyltransferase I (CPT-Ialpha) through the first intron. Biochim. Biophys. Acta 1679, 164–173.
Song, S., Attia, R.R., Connaughton, S., Niesen, M.I., Ness, G.C., Elam, M.B., Hori, R.T., Cook, G.A., Park, E.A., 2010. Peroxisome proliferator activated receptor alpha (PPARalpha) and PPAR gamma coactivator (PGC-1alpha) induce carnitine palmitoyltransferase IA (CPT-1A) via independent gene elements. Mol. Cell. Endocrinol. 30, 54–63.
Speakman, J.R., 2018. The evolution of body fatness: trading off disease and predation risk. J. Exp. Biol. 221 (Pt Suppl 1) (pii: jeb167254).
Speakman, J.R., Mitchell, S.E., 2011. Caloric restriction. Mol. Asp. Med. 32, 159–221.
Sucajtys-Szulc, E., Goyke, E., Korczynska, J., Stelmanska, E., Rutkowski, B., Swierczynski, J., 2009. Refeeding after prolonged food restriction differentially affects hypothalamic and adipose tissue leptin gene expression. Neuropeptides. 43, 321–325.
Sugden, M.C., Bulmer, K., Gibbons, G.F., Knight, B.L., Holness, M.J., 2002. Peroxisome-proliferator-activated receptor-alpha (PPARalpha) deficiency leads to dysregulation of hepatic lipid and carbohydrate metabolism by fatty acids and insulin. Biochem. J. 364, 361–368.
Szepesi, B., Epstein, M.G., 1977. Effect of repeated food restriction-refeeding on growth rate and weight. Am. J. Clin. Nutr. 30, 1692–1702.
Tan, S., Wen, J., Zhao, Z.J., 2017. The effects of warm temperature on energy metabolism, thermogenesis and body fat content in female striped hamsters. Acta Theriol. Sin. 37, 162–171.
Tartaglia, L.A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G.J., Campfield, L.A., Clark, F.T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Moore, K.J., Smutko, J.S., Mays, G.G., Wool, E.A., Monroe, C.A., Tepper, R.I., 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell 83, 1263–1271.
Vega, R.B., Huss, J.M., Kelly, D.P., 2000. The coactivator PGC-1 cooperates with peroxisome proliferatoractivated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Mol. Cell. Biol. 20, 1868–1876.
Vink, R.G., Roumans, N.J., Arkenbosch, L.A., Mariman, E.C., van Baak, M.A., 2016. The effect of rate of weight loss on long-term weight regain in adults with overweight and obesity. Obesity (Silver Spring) 24, 321–327.
Weigle, D.S., Duell, P.B., Connor, W.E., Steiner, R.A., Soules, M.R., Kuijper, J.L., 1997. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. J. Clin. Endocrinol. Metab. 82, 561–565.
Weindruch, R., Walford, R.L., Fligiel, S., Guthrie, D., 1986. The retardation of aging in mice by dietary restriction-longevity, cancer, immunity and lifetime energy-intake. J. Nutr. 116, 641–654.
Wen, J., Tan, S., Qiao, Q., Shi, L., Huang, Y., Zhao, Z., 2018. The strategies of behavior, energetic and thermogenesis of striped hamsters in response to food deprivation. Integr. Zool. 13, 70–83.
Widdowson, P.S., Upton, R., Henderson, L., Buckingham, R., Wilson, S., Williams, G., 1997. Reciprocal regional changes in brain NPY receptor density during dietary restriction and dietary-induced obesity in the rat. Brain Res. 774, 1–10.

Woods, S.C., Seeley, R.J., Porte Jr., D., Schwartz, M.W., 1998. Signals that regulate food intake and energy homeostasis. Science 280, 1378–1383.
Xu, Y., Elmquist, J.K., Fukuda, M., 2011. Central nervous control of energy and glucose balance: focus on the central melanocortin system. Ann. N. Y. Acad. Sci. 1243, 1–14.
Xu, X.M., Chi, Q.S., Cao, J., Zhao, Z.J., 2018. The effect of aggression I: the increases of metabolic cost and mobilization of fat reserves in male striped hamsters. Horm. Behav. 98, 55–62.
Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., Friedman, J.M., 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432.
Zhang, Y., Ma, K., Song, S., Elam, M.B., Cook, G.A., Park, E.A., 2004. Peroxisomal proliferator-activated receptorgamma coactivator-1 alpha (PGC-1 alpha) enhances the thyroid hormone induction of carnitine palmitoyltransferase I (CPT-I alpha). J. Biol. Chem. 279, 53963–53971.
Zhang, L.N., Mitchell, S.E., Hambly, C., Morgan, D.G., Clapham, J.C., Speakman, J.R., 2012. Physiological and behavioral responses to intermittent starvation in C57BL/6J mice. Physiol. Behav. 105, 376–387.
Zhao, Z.J., 2012. Effect of food restriction on energy metabolism and thermogenesis in striped hamster (*Cricetulus barabensis*). Acta Theriol. Sin. 32, 297–305.
Zhao, Z.J., Cao, J., 2009. Plasticity in energy budget and behavior in Swiss mice and striped hamsters under stochastic food deprivation and refeeding. Comp. Biochem. Physiol. 154A, 84–91.
Zhao, Z.J., Cao, J., Meng, X.L., Li, Y.B., 2010. Seasonal variations in metabolism and thermoregulation in the striped hamster (*Cricetulus barabensis*). J. Therm. Biol. 35, 52–57.
Zhao, Z.J., Zhu, Q.X., Chen, K.X., Wang, Y.K., Cao, J., 2013. Energy budget, behavior and leptin in striped hamsters subjected to food restriction and refeeding. PLoS One 8, e54244.
Zhao, Z.J., Chen, K.X., Liu, Y.A., Wang, C.M., Cao, J., 2014a. Decreased circulating leptin and increased neuropeptide Y gene expression are implicated in food deprivation-induced hyperactivity in striped hamsters, *Cricetulus barabensis*. Horm. Behav. 65, 355–362.
Zhao, Z.J., Liu, Y.A., Xing, Y.J., Zhang, M.L., Ni, X.Y., Cao, J., 2014b. The role of leptin in striped hamsters subjected to food restriction and refeeding. Zool. Res. 35, 262–271.
Zhao, Z.J., Chi, Q.S., Cao, J., Wang, D.H., 2014c. Seasonal changes of body mass and energy budget in striped hamster: the role of leptin. Physiol. Biochem. Zool. 87, 245–256.
Zhao, Z.J., Chi, Q.S., Liu, Q.S., Zheng, W.H., Liu, J.S., Wang, D.H., 2014d. The shift of thermoneutral zone in striped hamster acclimated to different temperatures. PLoS One 9, e84396.
Zhao, Y., Chen, L.B., Mao, S.S., Min, H.X., Cao, J., 2018. Leptin resistance was involved in susceptibility to overweight in the striped hamster re-fed with high fat diet. Sci. Rep. 8, 920.
Zhou, Y.J., Rui, L.Y., 2013. Leptin signaling and leptin resistance. Front Med. 2013, 207–222.