Effects of random food deprivation and refeeding on energy metabolism, behavior and hypothalamic neuropeptide expression in

Apodemus chevrieri

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Abstract: Maintaining adaptive control of behavior and physiology is the main strategy used by animals in responding to changes of food resources. To investigate the effects of random food deprivation (FD) and refeeding on energy metabolism and behavior in *Apodemus chevrieri*, we acclimated adult males to FD for 4 weeks, then refed them *ad libitum* for 4 weeks (FD-Re group). During the period of FD, animals were fed *ad libitum* for 4 randomly assigned days each week, and deprived of food the other 3 days. A control group was fed *ad libitum* for 8 weeks. At 4 and 8 weeks we measured body mass, thermogenesis, serum leptin levels, body composition, gastrointestinal tract morphology, behavior and hypothalamic neuropeptide expression. At 4 weeks, food intake, gastrointestinal mass, neuropeptide Y (NPY) and agouti-related protein (AgRP) mRNA expressions increased and thermogenesis, leptin levels, pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) expressions decreased in FD compared with controls. FD also showed more resting behavior and less activity than the controls on *ad libitum* day. There were no differences between FD-Re and controls at 8 weeks, indicating significant plasticity. These results suggested that animals can compensate for unpredictable

1 reduction in food availability by increasing food intake and reducing energy expended through
2 thermogenesis and activity. Leptin **levels**, NPY, AgRP, POMC, and CART mRNA levels may
3 also regulate energy metabolism. Significant plasticity in energy metabolism and behavior was
4 shown by *A. chevrieri* over a short timescale, allowing them to adapt to food shortages in
5 nutritionally unpredictable environments.

6 **Key words:** *Apodemus chevrieri*; behavioral patterns; hypothalamic neuropeptide; serum leptin
7 levels

8 **1. Introduction**

9 Physiological adaptability, including adjustments of body mass and energy metabolism, is
10 one of the main strategies used by animals to respond to fluctuations in their natural environment,
11 significantly enhancing their ability to survive (Zhao and Wang, 2007). Availability of food
12 resources **affect** animal survival, reproduction and evolution (Bacigalupe and Bozinovic, 2002).
13 Faced with food restrictions, deprivation or hunger, small rodents may reduce body mass, body
14 temperature, energy expenditure, thermogenesis and/or begin to store food (Zhan et al., 2009).
15 Many studies **suggested** that small mammals adapt to food shortage through adjustments in body
16 mass and energy balance, and food restriction has obvious effects on physiological characteristics,
17 including reductions in energy consumption and changes to their digestive tracts (Bozinovic et al.,
18 2007). Food restriction **reduced** body mass, body fat mass, metabolic rate and serum leptin levels
19 in *Lasiopodomys brandtii* (Zhan et al., 2009) and random food deprivation **reduced** body mass,
20 thermogenesis in *Eothenomys miletus* (Zhu et al., 2014). However, while body mass in rats and
21 mice increased significantly after re-feeding (Friedman and Halaas, 1998; Woods et al., 1998), but
22 *Mesocricetus auratus* (Schneider et al., 2000) and *Phodopus sungorus* (Day and Bartness, 2003)

1 did not increased body mass after refeeding. The phenotype of digestive tracts can reflect
2 physiological and ecological adaptations made by animals to their environment (Pucek, 1965).
3 Food restrictions have been shown to induce changes in the morphology of the digestive tract
4 (Bozinovic, 2007).

5 Resting metabolic rate (RMR) and nonshivering thermogenesis (NST) are two important
6 modes of energy expenditure in small mammals, and are greatly influenced by environmental
7 factors (Zhao and Wang, 2007). Changes in food quantity and/or quality lead to variations in
8 metabolic energy expenditure (Veloso and Bozinovic, 1993), and many studies have demonstrated
9 that the low quality food reduced RMR in rodents (Hambly and Speakman, 2005; Zhao and Wang,
10 2007). Many animals also actively or passively regulate behavior to adapt to changes in
11 environmental conditions. In response to food restriction, *Mus musculus* (Blank and Desjardins,
12 1985) and rats (Sherwin, 1998) increased their activity levels, while MF1 mice (Hambly and
13 Speakman, 2005) and *Eutamias minimus* (Cornish and Mrosovsky, 1965) reduced activity levels,
14 suggesting that behavioral responses to food restriction can be species-specific.

15 Leptin, a 16-kDa protein, contributes to the maintenance of body mass by influencing food
16 intake and energy expenditure (Friedman and Hallas 1998). A positive correlation has been
17 observed between serum leptin levels and body fat mass in many small mammals (Abelenda et al.
18 2003). The hypothalamic arcuate nucleus (ARC) can regulate food intake in response to
19 environmental changes (Heijboer et al., 2005; Aguilar et al., 2011). Within the ARC are two types
20 of neuropeptides: orexigenic neuropeptides including neuropeptide Y (NPY) and agouti-related
21 protein (AgRP); and anorectic neuropeptides including pro-opiomelanocortin (POMC) and
22 cocaine- and amphetamine-regulated transcript (CART). The balance between orexigenic and

1 anorectic neuron activities can influence food intake and energy expenditure in mammals
2 (Friedman and Halaas, 1998). Leptin is mediated by a hierarchy of orexigenic and anorectic
3 neuropeptidergic neurons located in specific sites in the hypothalamus (Arch, 2005). In a state of
4 negative energy balance, a reduction in leptin stimulates NPY and AgRP expressions and
5 suppresses POMC and CART expressions, eliciting an increase in food intake and a reduction in
6 energy expenditure (Xiao et al., 2005). Studies showed that when rats are fed with a high fat food,
7 NPY expression and body mass are increased (Toshihiro and Akio, 1993). Prolonged hunger also
8 increases NPY expression in rats (Sucajty-Szulc et al., 2008); and food-restricted lactating rats
9 have higher NPY levels than those fed *ad libitum* (Abizaid et al., 1997). Prolonged food restriction
10 reduces body mass, body fat mass, POMC and CART expressions, and increases AgRP
11 expressions in sheep (Henry et al., 2001). *Phodopus sungorus* showed increased AgRP expression
12 during a food shortage (Mercer et al., 2000). Fasting can also reduce CART expression (Tian et al.,
13 2004), for instance mice showed reduced CART expression and increased appetite following a
14 48-hour fast (Yoo et al., 2011).

15 Chevrier's field mouse, *Apodemus chevrieri* (Mammalia: Rodentia: **Muridae**) is endemic to
16 the Hengduan mountains region of China (Zheng 1993). *A. chevrieri* has been reported to show
17 seasonal changes in body mass and digestive tract morphology (Zhu et al., 2012). During cold
18 exposure, *A. chevrieri* showed increased energy intake, thermogenesis and reduced body mass,
19 and serum leptin levels (Zhu et al., 2011). Food restriction alone may also reduce body mass in *A.*
20 *chevrieri* (Zhu et al., 2013). However, the effects of random food deprivation (FD) on serum leptin
21 levels, behavior, hypothalamic neuropeptide expression, and their influence on body mass
22 regulation and thermogenesis in *A. chevrieri* remain unknown. In the present study, we examined

the effects of FD and refeeding on energy metabolism and behavior in *Apodemus chevrieri* adult males, we measured body mass, thermogenesis, serum leptin levels, body composition, gastrointestinal tract mass and length, behavior and hypothalamic neuropeptide expression after 4 weeks of FD and again after 4 weeks of refeeding. We hypothesized that *A. chevrieri* would respond to FD by reducing body mass, body fat mass, and thermogenesis in association with reduced serum leptin levels, but energy metabolism would return to normal after refeeding, demonstrating phenotypic plasticity. We predicted that leptin levels, NPY, AgRP, POMC, and CART mRNA levels would play a role in the regulation of energy metabolism in *A. chevrieri*.

2. Materials and Methods

2.1 Animals and sampling

A. chevrieri were obtained from a laboratory colony, founded by animals captured from farmland (26°15′–26°45′N; 99°40′–99°55′E; altitude 2,590m) in Jianchuan County, Yunnan province. Adult male *A. chevrieri* (120 days of age) were housed individually in plastic boxes (26×16×15cm³). Animals were kept in a room temperature of 25±1 °C with a photoperiod of 12L:12D (with lights on at 08:00 h), and provided food (standard rabbit chow produced by Kunming Medical University, Kunming) and water *ad libitum*. After 1 month of stabilization, animals were randomly assigned to a control group or a random food deprivation refeeding group (FD-Re). Controls (n=20), were fed *ad libitum* during 8 weeks, while FD-Re (n=20) were deprived of food for a random 3 days each week and fed *ad libitum* on the other 4 days for a total of 4 weeks, then fed *ad libitum* for a further 4 weeks (Fig 1) (Barboza et al., 2001; 2002). After 4 weeks, 10 animals were randomly selected from each group (4W Control group and 4W FD group)

1 for the measurement of RMR, NST, serum leptin levels, body composition, gastrointestinal tract
2 mass and length, behavior and hypothalamic neuropeptide expression. These measurements were
3 taken again from the remaining 10 animals of each group (8W Control **group** and 8W FD-Re
4 group) at 8 weeks. All animals were sacrificed between 0900 h and 1100 h by decapitation, and
5 animals were dissected to evaluate organ morphology. Blood was centrifuged at 4,000 rpm for 30
6 min after a 30 min interval. Blood serum was collected and stored at -75°C prior to hormone
7 measurement. During the 8 weeks of experiment, food intake was measured daily and body mass
8 was measured in 3 days. All animals were dissected to evaluate organ morphology, and young
9 individuals were excluded in present study. All animal procedures were approved by the Animal
10 Care and Use Committee of Life of Science, Yunnan Normal University (13-0901-011).

11 *2.2 Morphology*

12 Immediately after sacrifice at 4 and 8 weeks, trunk blood was collected and visceral organs
13 including liver, brown adipose tissue (BAT), heart, lung, kidneys, spleen and gastrointestinal tract
14 (stomach, small intestine, caecum, large intestine) were excised and weighed (± 1 mg). The
15 stomach and intestines were rinsed with saline to remove gut contents and then weighed. The
16 remaining carcass and the excised organs were dried to constant mass in an oven at 60°C (for at
17 least 72 h), and then weighed again to obtain a dry mass. Total body fat **mass** was extracted from
18 the dried carcass by ether extraction in a Soxhlet apparatus (Zhang and Wang 2007).

19 *2.3 Food intake*

20 Food intake was measured following Rosenmann et al. (2003). Briefly, animals were kept in

1 metabolic cage ($20 \times 15 \times 15 \text{ cm}^3$) with no nest materials, and fed standard rabbit chow. Animals
2 were fed at a set time (at 1100 h), and residual food collected was collected the following day.
3 Residual food was dried in a vacuum dryer to a constant weight, which was recorded.

4 *2.4 Measurement of metabolic rates*

5 Metabolic rates were measured using an AD ML870 open respirometer (AD Instruments,
6 Australia) at 25°C within the thermal neutral zone, and gas analysis was performed using a
7 ML206 gas analysis instrument (AD Instruments). The temperature was controlled using a
8 SPX-300 artificial climatic incubator ($\pm 0.5^\circ\text{C}$) (Changsha, China), the metabolic chamber volume
9 was 500ml and airflow rate was 200 ml/min. Animals were stabilized in the metabolic chamber
10 for at least 60 min prior to the RMR measurement, and oxygen consumption was recorded for at
11 least 120 min at 1 min intervals. Ten stable consecutive low readings were taken to calculate RMR
12 following Li and Wang (2005), using the method for calculating the metabolic rate provides by
13 Hills (1972).

14 Nonshivering thermogenesis (NST) was induced by a subcutaneous injection of
15 norepinephrine (NE) (Shanghai Harvest Pharmaceutical Co. Ltd, China) and measured at 25°C .
16 Two consecutive high oxygen consumption readings from each 60-min measurement were taken
17 to calculate NST (Li and Wang, 2005). The doses of norepinephrine were approximately 0.8-1.0
18 mg/kg, according to dose-dependent response curves generated before the experiment and using
19 the equation of Heldmaier (1971).

20 *2.5 Behavioral observations*

21 Behaviors were observed on day 27 (FD food deprivation day), day 28 (FD feeding day) and

1 day 56. The dominant behavior was classified into one of four categories: general activity, feeding
2 behavior, grooming behavior and resting behavior (Speakman et al. 2001). General activity
3 denoted any active **movements** such as walking around the cage and climbing on the cage bars,
4 feeding behavior denoted eating but not drinking; grooming denoted self-grooming and resting
5 denoted **inactivity** in any area of the cage. Animals were observed in sequence for 30 s each over
6 the course of an 8-min session, during which the dominant behavior was recorded as one of the
7 four aforementioned types. Observation was made during period of 0800–1200 h and 1800–2200
8 h, respectively. Each animal was observed 60 times in 8 h and the percentage time spent on each
9 behavior was calculated for each animal (Speakman et al. 2001). Observations during the dark
10 phase were made using a 30 W red light (Zhao and Cao 2009).

11 *2.6 Measurement of serum leptin levels*

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

16 *2.7 Measurements of hypothalamic neuropeptide gene expression*

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

1 We designed species-specific primers for these genes based on the cloned gene sequences of
2 *A. chevrieri*. Species-specific primers were designed in real-time q-PCR for β -actin and the four
3 hypothalamic genes are shown in Table 1 and verified effectively in *A. chevrieri* (Zhang et al.,
4 2015). Standard curves were constructed for each gene using serial dilutions of cDNA (1 to
5 26-fold dilutions). A comparison of the standard curves of the target genes and β -actin revealed
6 that they had similar amplification efficiencies, validating the comparative quantity method used.
7 Real-time q-PCR was performed using a SYBR Green I qPCR kit (Invitrogen) and the ABI
8 Prism[®] 7000 Sequence Detection system (Applied Biosystems, Carlsbad CA, USA). Real-time
9 qPCR was carried out in 20- μ L reactions comprising 9.5 μ L RNase-free ddH₂O, 9.0 μ L Platinum[®]
10 Quantitative PCR SuperMix-UDG (including Rox), 0.5 μ L cDNA templates, 0.5 μ L 10 μ mol/L
11 forward primer, and 0.5 μ L 10 μ mol/L reverse primer. Each sample was analyzed in triplicate.
12 Thermal cycling conditions were: 50 °C for 120 s, 95 °C for 120 s, 45 cycles of 95 °C for 15 s,
13 and 60 °C for 45 s.

14 **Table 1 Gene-Specific Primers Used for Real-Time qPCR.**

Primer	Oligonucleotide sequence (5' to 3')	Product size (bp)
NPY (forward)	TCGCTCTGTCCCTGCTCGTGTG	130
NPY(reverse)	TCTCTTGCCGTATCTCTGCCTGGTG	
AgRP(forward)	AGAGTTCTCAGGTCTAAGTCT	190
AgRP(reverse)	CTTGAAGAAGCGGCAGTAGCACGT	
POMC(forward)	CCTGTGAAGGTGTACCCAATGTC	241
POMC(reverse)	CACGTTCTTGATGATGGCGTTC	
CART(forward)	AGAAGAAGTACGGCCAAGTCC	50
CART(reverse)	CACACAGCTTCCCGATCC	
β -actin(forward)	TCGCTCTGTCCCTGCTCGTGTG	170
β -actin (reverse)	TCTCTTGCCGTATCTCTGCCTGGTG	

2.8 Statistical analysis

Data were analyzed using the software package SPSS 15.0. Prior to all statistical analyses, data were examined for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene tests, respectively. Body mass and food intake were analyzed using repeated measures ANOVA. RMR, NST, body composition and hypothalamic neuropeptide gene expression were analyzed using One-way ANCOVA, with body mass or carcass mass as a covariate, followed by Tukey's HSD post-hoc tests. Categorized behavioral data were analyzed using independent-samples t-test, with an arcsine-square-root transformation performed on the percentage data prior to analysis. To detect any associations of serum leptin levels with body fat mass, we used Pearson-correlation analysis. Results are presented as means $\pm$ SEM and $P < 0.05$ was considered to be statistically significant.

3. Result

3.1 Body mass

There were no differences in body mass between control and FD-Re groups on day 0 ($t=0.12$, $P>0.05$). On day 28, body mass was significantly reduced in the FD-Re group compared with **that** **of** controls (FD-Re: 28.32 ± 0.32 g, control: 33.72 ± 0.41 g, $t=4.25$, $P<0.01$). On day 56, body mass in 8W FD-Re group had returned to control levels, with no significant differences between **two** groups ($t=0.024$, $P>0.05$, Fig 2).

3.2 Body composition and gastrointestinal tract mass and length

FD had a significant effect on carcass mass, body fat mass, wet liver mass and wet BAT mass by day 28 (Table 2), but **had** no effect on our other measures of body composition ($P>0.05$). FD also had a significant effect by day 28 on the mass of the gastrointestinal tract with gut content and

1 the length of small intestine, but did not affect other gastrointestinal tract measurements ($P>0.05$,
2 Table 3).

3 *3.3 Food intake*

4 There were no differences of food intake between control and FD-Re groups on day 0 ($t=0.25$,
5 $P>0.05$). During the 4 weeks of FD, food intake in 4W FD group increased significantly ($t=-5.65$,
6 $P<0.01$). During the subsequent 4 weeks of refeeding, FD-Re food intake decreased, returning to
7 the control levels on day 34 ($t=0.19$, $P>0.05$). Food intake showed significantly changes with time
8 in FD-Re group ($F=26.32$, $P<0.01$, Fig 3), and no differences with time in the control group
9 ($F=0.68$, $P>0.05$, Fig 3).

10 *3.4 RMR and NST*

11 On day 28, RMR was 13.93% lower in the FD-Re group than **that of** the control group
12 (FD-Re: 2.41 ± 0.11 mlO₂/g.h, control: 2.80 ± 0.14 mlO₂/g.h), but returned to control levels by
13 day 56 ($F=2.43$, $P<0.05$, Fig 4). NST was also reduced in the FD-Re group at day 28, but returned
14 to control levels by day 56 ($F=2.56$, $P<0.05$, Fig 5).

15 *3.5 Behavior*

16 On day 27 (a food deprivation day), the percentage of time spent on general activity and
17 resting was greater in the FD-Re group than **that of** the control group (activity: $t=3.21$, $P<0.01$;
18 resting: $t=1.74$, $P<0.05$), and that spent on grooming was lower in FD-Re group than that in
19 control group ($t=-2.15$, $P<0.05$, Fig 6A). On day 28 (feeding day), the percentage of time spent
20 feeding and resting behavior were higher in FD-Re group than that in control group (feeding:
21 $t=2.27$, $P<0.05$; resting: $t=3.02$, $P<0.01$), and that spent activity and grooming was lower in FD-Re
22 group than that in control group (activity: $t=-4.35$, $P<0.01$; grooming: $t=-6.98$, $P<0.01$, Fig 6B).

On day 56, there were no differences in the percentage time spent on different behaviors between the two groups ($P>0.05$, Fig 6C).

3.6 Serum leptin level and hypothalamic neuropeptide gene expression

Serum leptin levels was reduced in the FD-Re group at day 28 compared with controls (FD-Re: 0.98 ± 0.03 ml O_2 /g.h, controls: 1.51 ± 0.06 ml O_2 /g.h), but returned to control levels by day 56 ($F=2.13$, $P<0.05$, Fig 7). There was a positive correlation between body fat mass and serum leptin levels ($r=0.625$, $P<0.01$). FD had significant effects on NPY, AgRP, POMC and CART expressions (NPY: $F=2.25$, $P<0.05$; AgRP: $F=3.36$, $P<0.01$; POMC: $F=3.19$, $P<0.01$; CART: $F=2.85$, $P<0.01$, Fig 8). NPY and AgRP expressions were increased and POMC and CART expressions were reduced on day 28 in FD-Re group compared with controls, but returned to control levels on day 56.

4. Discussion

4.1 Body mass and body composition

Body mass is an important index of animal nutritional status, and its stability relies on energy balance (Zhao and Wang, 2007). In the present study, FD reduced body mass significantly in *A. chevrieri*, which returned to control levels after refeeding, as shown by other rodent models (Trayhurn and Jennings, 1988; Kouda et al., 2004). The reduction in body mass was associated with reduced carcass mass and body fat mass (Mustonen et al., 2005). With the exception of wet liver mass and BAT mass, organ mass was not affected by FD, suggesting that *A. chevrieri* maintain energy expenditure by reducing carcass mass, liver and BAT mass use. After refeeding, carcass mass and body fat mass returned to control levels, which probably contributed to the recovery of body mass (Freminet, 1981).

4.2 Food intake

Small mammals, such as *Meriones shawi*, usually increase food intake under conditions of food restriction, (Demas and Bartness, 1999). When faced with large environmental fluctuations in food quality and/or quantity, increasing food intake can improve their survival ability and reduce their risk of predation, though some animals do not use this strategy. In our study, food intake increased significantly in the FD-Re group compared with the control group during the period of FD, but returned to the control levels after refeeding. The digestive tract also showed significant changes with FD, with greater small intestine length and greater digestive tract mass with gut content in the FD group than that of control group. These results indicated that *A. chevrieri* can make full use of occasional feeding opportunity by increasing food intake to compensate for reduced food availability. Similar results have also been reported for other animals (Naya et al. 2005; 2008; Zhao and Wang 2007). The return of food intake to control levels after refeeding suggests that digestive tract physiological plasticity plays an important roles in body mass regulation in animals (Zhao et al., 2009).

4.3 BMR and NST

RMR and NST consume significant amounts of energy in small mammals, which can reflect overall levels of energy consumption in different species or individuals. RMR and NST are affected by temperature, food, the activity levels as well as other factors (Terblanche et al., 2007). In the present study, FD significantly reduced RMR and NST. This suggests that food deprived *A. chevrieri* could not consume enough energy to meet their requirements, so RMR and NST were lowered to reduce energy consumption to maintain the body's normal physiological activities. Hunger has previously been shown to reduce the metabolic rate in rats and mice (DeLaney et al.,

1999). Liver is the major organ for RMR, and BAT is the major organs for NST (Himms-Hagen,1990). In the present study, the reduction of RMR and NST in FD group may be affected by reduced liver and BAT mass. RMR and NST returned to the control levels after refeeding, as did liver and BAT mass, demonstrating remarkable plasticity.

4.4 Behavior

Animals often change their behavior to adapt to **changes** in environmental conditions (Zhao and Cao 2009). In the present study, the level of general activity was significantly higher in FD group than **that of** controls on a food deprivation day, as also shown in mice (Sherwin 1998), indicating that animal increase activity to look for food when the food resources are limited (Alvarenga et al. 2005). On *ad libitum* day, FD group **animals** spent a greater percentage of time feeding than controls, suggesting that animal can reduce general activity, increased feeding behavior and reduce energy expenditure to compensate for a shortage of food (Gursoy et al. 2001). Moreover, our results indicated that on an *ad libitum* feeding day, FD-Re animals reduced their energy expenditure by reducing general activity and increasing resting behavior, and increased their feeding behavior to increase **food** intake. After refeeding, the percentage time spent on activity, grooming, feeding and rest behavior all returned to the control levels in the FD-Re group, showing significant plasticity and suggesting that this plasticity of behavior plays an important role in the adaptation of small mammals to food shortages.

4.5 Serum leptin level and hypothalamic neuropeptide gene expression

Leptin plays an important role in body mass regulation (Friedman and Hallas 1998), which suggests that leptin levels can reflect content of body fat mass (Schneider et al., 2000). We found a positive correlation between serum leptin levels and body fat mass in the present study, supporting

the claim that leptin can act as a lipid signaling molecule (Zhao and Wang, 2006). **Orexigenic neuropeptides and anorectic neuropeptides can regulate food intake in small mammals** (Arch, 2005). In the present study, FD had significant effects on hypothalamus neuropeptide **genes expressions** in *A. chevrieri*. NPY and AgRP **expressions** were increased to increase appetite and reduce heat production. POMC and CART **expressions** were reduced, which also increased appetite (Balaskó et al., 2006; Larsen and Hunter, 2006; Kim and Harrington, 2008; Tracy et al., 2008; Lukaszewski et al., 2013; Zhao et al., 2014). These results indicated that NPY/AgRP and POMC/CART **expressions can regulate** body mass and energy metabolism in *A. chevrieri* under conditions of FD.

In conclusion, FD had significant effects on body mass, food intake, behavior, gastrointestinal tract mass and length and hypothalamic neuropeptide gene expression in *A. chevrieri*. FD group had a significantly higher percentage of feeding behavior and lower activity than control group on *ad libitum* day. These effects were no longer apparent after 4 weeks of *ad libitum* refeeding, indicating significant plasticity. Our results suggest that animals can compensate for an unpredictable reduction in food availability by increasing food intake and reducing energy expended through thermogenesis and activity. Changes in leptin **levels**, NPY, AgRP, POMC, and CART mRNA levels may play a role in regulating energy metabolism .

Acknowledgments

This research was financially supported by National Science Foundation of China (No. 31260097; 31560126), and Launch scientific research projects of Yunnan Normal University. **We wish to thank Pro. Burkart Engesser at Historisches Museum Basel, Switzerland for correcting the English usage in the draft.** Thank you for the two anonymous reviewers and the editor of the journal for their valuable comments.

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Fig 1 A time line showing which days food deprivation occurred and which day each measurement was taken in *Apodemus chevrieri*. An asterisk put below every food deprived day. "R", refeeding.

Fig 2 Changes in body mass of *Apodemus chevrieri* in response to random food deprivation and *ad libitum* re-feeding.

Fig 3 Changes in food intake of *Apodemus chevrieri* in response to random food deprivation and *ad libitum* re-feeding.

Fig 4 Changes in RMR of *Apodemus chevrieri* in response to random food deprivation and *ad libitum* re-feeding. Different superscripts in each row indicate significant difference (P<0.05).

Fig 5 Changes in NST of *Apodemus chevrieri* in response to random food deprivation and *ad libitum* re-feeding. Different superscripts in each row indicate significant difference (P<0.05).

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Fig 6 Changes in general activity, feeding, grooming and resting behavior of *Apodemus chevrieri* on food deprived day (A), on *ad libitum* day (B) and on re-feeding day (C). * $P < 0.05$, ** $P < 0.01$

Fig 7 Changes in serum leptin levels of *Apodemus chevrieri* in response to random food deprivation and *ad libitum* re-feeding. Different superscripts in each row indicate significant difference ($P<0.05$).

Fig 8 Changes in NPY, AgRP, POMC and CART expression of *Apodemus chevrieri* in response to random food deprivation and *ad libitum* re-feeding. Different superscripts in each row indicate significant difference ($P<0.05$).

Table 2 Effects of food deprivation and *ad libitum* re-feeding on body composition in *Apodemus chevrieri*.

Parameters	4W Control	4W FD group	8W Control	8W FD-Re group
Body mass(g)	33.72±0.22 ^a	28.32±0.19 ^b	33.75±0.25 ^a	33.77±0.21 ^a
Wet Carcass mass(g)	23.61±0.22 ^a	20.36±0.17 ^b	24.51±0.15 ^a	23.45±0.18 ^a
Dry carcass mass(g)	7.89±0.21 ^a	6.84±0.12 ^b	8.01±0.19 ^a	7.91±0.17 ^a
Body fat mass(g)	4.56±0.12 ^a	2.42±0.09 ^b	4.71±0.13 ^a	4.51±0.10 ^a
Heart				

Wet mass(g)	0.256±0.013 ^a	0.238±0.012 ^b	0.249±0.008 ^a	0.234±0.009 ^a
Dry mass(g)	0.061±0.003	0.058±0.006	0.063±0.002	0.060±0.003
Lungs				
Wet mass(g)	0.224±0.016	0.218±0.012	0.228±0.011	0.221±0.009
Dry mass(g)	0.064±0.004	0.061±0.002	0.059±0.003	0.058±0.004
Liver				
Wet mass(g)	2.231±0.142	1.672±0.126	2.241±0.153	2.204±0.114
Dry mass(g)	0.876±0.024	0.842±0.015	0.861±0.013	0.859±0.011
Brown adipose tissue				
Wet mass(g)	0.214±0.013 ^a	0.152±0.009 ^b	0.211±0.012 ^a	0.198±0.011 ^a
Dry mass(g)	0.019±0.001	0.016±0.001	0.020±0.001	0.018±0.001
Kidney				
Wet mass(g)	0.452±0.026	0.421±0.021	0.461±0.019	0.435±0.016
Dry mass(g)	0.136±0.005	0.122±0.006	0.139±0.008	0.129±0.008
Spleen				
Wet mass(g)	0.059±0.002	0.061±0.003	0.064±0.003	0.058±0.002
Dry mass(g)	0.015±0.001	0.013±0.001	0.016±0.001	0.015±0.001

1 Different superscripts in each row indicate significant difference (P<0.05).

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3 Table 3 Effects of food deprivation and *ad libitum* re-feeding on gastrointestinal tract in *Apodemus chevrieri*.

Parameters	4W Control	4W FD group	8W Control	8W FD-Re group
Stomach				
Length(mm)	22.71±0.126	23.60±0.152	22.61±0.162	22.19±0.146
Mass with content(g)	0.613±0.162 ^b	0.861±0.135 ^a	0.621±0.122 ^b	0.638±0.146 ^b
Wet mass(g)	0.251±0.019	0.259±0.018	0.272±0.014	0.260±0.012
Dry mass(g)	0.064±0.003	0.065±0.002	0.063±0.002	0.061±0.004
Small intestine				
Length(mm)	356.21±23.21 ^b	370.16±19.87 ^a	361.25±21.26 ^b	365.23±23.69 ^b
Mass with content(g)	1.123±0.102 ^b	1.452±0.095 ^a	1.095±0.087 ^b	1.114±0.065 ^b
Wet mass(g)	0.451±0.016	0.463±0.011	0.459±0.021	0.456±0.019
Dry mass(g)	0.089±0.002	0.091±0.002	0.085±0.003	0.088±0.002
Large intestine				
Length(mm)	184.23±12.36	192.32±18.32	186.65±13.02	187.54±14.65
Mass with content(g)	0.325±0.019 ^b	0.474±0.026 ^a	0.326±0.022 ^b	0.331±0.025 ^b
Wet mass(g)	0.221±0.011	0.236±0.016	0.219±0.009	0.223±0.012
Dry mass(g)	0.049±0.003	0.052±0.002	0.048±0.004	0.051±0.003
Cecum				
Length(mm)	96.32±6.54	98.25±5.62	93.98±6.58	94.63±6.31
Mass with content(g)	0.865±0.033 ^b	0.984±0.035 ^a	0.859±0.024 ^b	0.864±0.021 ^b
Wet mass(g)	0.286±0.016	0.291±0.025	0.276±0.021	0.284±0.013
Dry mass(g)	0.051±0.002	0.053±0.003	0.049±0.004	0.051±0.004

4 Different superscripts in each row indicate significant difference (P<0.05).