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Effects of Temperature and Diet on Energy Budget and Hormone Concentrations in South China Field Mouse, *Apodemus draco*, from Hengduan Mountain Region

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Abstract.- Environmental factors play important roles in seasonal adaptation of thermogenesis and hormone concentrations in small mammals. To investigate the roles of temperature and diet in the regulation of energy budget and thermogenesis, adult male *Apodemus draco* were acclimatized to one of 4 groups: 1) cold and low fat diet; 2) cold and high fat diet; 3) warm and low fat diet; 4) warm and high fat diet. After a 7-week acclimation, we determined body mass, energy inatke, resting metabolic rate (RMR), nonshivering thermogenesis (NST), digestive tract morphology, serum leptin level, triiodothyronine (T_3), thyroxine (T_4) concentrations, mitochondrial protein content (MP), cytochrome c oxidase (COX) activity in liver and brown adipose tissue (BAT), and uncoupling protein 1 (UCP1) content in BAT. The results showed that cold induced decreases in body mass, body fat mass and serum leptin level, and increases in RMR, NST, COX activity, UCP l content and T_3 concentrations. There were no significant effects of diets varying in fat content on body mass, RMR, NST, UCP1 content and serum leptin level. However, high-fat diet significantly reduced energy intake, mass with content and wet mass of the total digestive tract, and elevated digestibility. Serum leptin level was positively correlated with body fat mass, however, not with energy intake. Our results suggested that *A. draco* could resist high-fat diet induced obesity, which might be mediated by the increased sensitivity of leptin and enhanced thermogenesis during cold acclimation. It indicated *A. draco* could prevent excessive obesity by adaptive regulation of energy metabolism and thermogenesis.

Keywords: *Apodemus draco*, high fat diet, thermogenesis, body mass, leptin, triiodothyronine (T_3), thyroxine (T_4)

INTRODUCTION

Complex phenotypic traits are emergent characters that arise from multiple systems, such as behavior and thermogenesis performance. To know how these reactions respond to changing environmental conditions are particularly challenging, because responses encompass several levels of organization involving multiple physiological functions (Rezende *et al*., 2009). Animals living in a seasonal environment experience constantly changing climate and food availability (Larcombe and Withers, 2008; Scherbarth and Steinlechner, 2010), some winter-active rodent species showed reduction in body mass and body fat mass and increase in resting metabolic rate (RMR) and nonshivering thermogenesis (NST) (Chen *et al*., 2012), such as Swiss mice (Zhao *et al*., 2010a), *Microtus maximowiczii* (Chen *et al*., 2012), *Phodopus sungorus* (Braulke *et al*., 2010), *Alligator mississippiensis* (Boggs *et al*., 2011), *Eothenomys miletus* (Zhu *et al*., 2010a,b) and *Apodemus chevrieri* (Zhu *et al*., 2011). Body mass was also affected by food quality, digestibility or availability (Zhao *et al*., 2010b). Body mass and body fat content increased significantly in rats fed with high fat diet (Posey *et al*., 2009; Jones *et al*., 2009). However, in some mammals, for example *Meriones shawi* and *M. pennsylvanicus*, high fat diet did not induce increase in body mass and body fat content (Mcelroy *et al*., 1986; El-Bakry *et al*., 1999).

NST is an important mechanism for cold-exposed small mammals to generate heat (Jansky, 1973). Brown adipose tissue (BAT) is the main site of NST (Ricquier and Bouillaud, 2000). NST in small mammals is originated principally through activation of uncoupling protein-1 (UCP1) (Wang *et al*., 2006). UCP1 is a 32-kD carrier protein, which renders the inner membrane of the mitochondria ‘‘leaky’’ and hence releases energy in the form of heat rather than storing it as ATP (Krauss *et al*.,

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2005). The cold-induced increase in BAT UCP1 content was also found in Siberian hamsters (*Phodopus sungorus*) (Von *et al*., 2001) and *Spermophilus dauricus* (Li *et al*., 2001). As the terminal enzyme in oxidative phosphorylation in mitochondria, cytochrome c oxidase (COX, complex IV) is involved in mitochondrial energy metabolism (Kadenbach *et al*., 2000).

Thyroid hormones (THs) are necessary for the proper development and regulation of immune, reproductive, and metabolic systems in vertebrates. There are two major forms of THs found in circulation. Thyroxine (T_4) is the prohormone, which has four molecules of iodide. T_4 is present at higher circulating concentrations when compared to the highly active form, triiodothyronine (T_3), which has three iodide molecules (Boggs *et al*., 2011). It has also been demonstrated that T_3, T_4 affect adaptive thermogenesis by influencing several aspects of energy metabolism (Krotkiewski, 2002; Zhu *et al*., 2010b). Leptin, a hormone primarily synthesized and secreted from adipose tissue, is known to regulate both food intake and body mass (Friedman and Halaas, 1998). Decreased plasma leptin is accompanied by hyperphagia in cold-exposed rats (Bing *et al*., 1998). The discovery of leptin has improved our understanding of the relationship between adipose tissue and energy homeostasis (Zhang *et al*., 1994). For example, many small mammals showed seasonal fluctuations of energy intake, body mass and body fat content with the change of serum leptin level (Klingenspor *et al*., l996, 2000; Li and Wang, 2005; Wang *et al*., 2006). But there was a lack of researches about effects of the high fat diet on NST, BAT UCP1 content as well as serum leptin level, especially in wild species (Mcelroy *et al*., 1986; Dark and Zuker, 1986; El-Bakry *et al*., 1999; Zhao *et al*., 2010b).

The Hengduan Mountains region is located at the boundary between the Palaearctic and Oriental zones, which is characterized by alpine and gorge areas. It has abundant mammals and has been considered “a refuge during the fourth ice age” (Zhu *et al*., 2010a). Small mammals would be expected to show specific physiological and ecological adaptations to the particular geographical and climatic features of this region. Now only a few studies about *A. draco* were reported (Li *et al*., 2009; Zhu *et al*., 2012; 2013a,b). Therefore, we determined effect of cold temperature and high-fat diet on body mass, body fat mass, body compositions, energy intake and BAT UCP1 content to test the hypothesis that cold acclimation induced decreases in body mass and body fat mass in *A. draco*, and *A. draco* thus may be resistant to the high-fat diet-induced obesity. We predict that *A. draco* would show physiological regulations in energy balance and hormone concentrations under different temperature and food quality.

MATERIALS AND METHODS

Samples

A. draco were obtained from a captive population started from approximately 100 animals captured in farmland (26°15´~26°45´N; 99°40´~99°55´E; altitude 2,590m) in Jianchuan County, Yunnan province, 2010. Mean annual temperature 9.1^{o}C, average temperatures in January and July was -4.0^{o}C and 24.1^{o}C, respectively. *A. draco* were breed for two generations in School of life Science of Yunnan Normal University, park in plastic box(260mm×160mm×150mm), one in a box without any bedding material, and were maintained at the room temperature of 25±1°C, under a photoperiod of 12L:12D (with lights on at 04:00). *A. draco* (about 120 days) were allowed to acclimate to these conditions for 4 weeks. Food (normal diet: rabbit pellet chow; Kunming Medical University) and water were provided *ad libitum*. All pregnant, lactating or young individuals were excluded. All animal procedures were licensed under the Institutional Animal Care and Use Committee of the Institute of Zoology, Yunnan Normal University.

Following the acclimation period, adult male *A. draco* were then randomly divided into four groups (n=10 in each group): 1) cold and low fat diet, CL; 2) cold and high fat diet, CH; 3) warm and low fat diet, WL; 4) warm and high fat diet, WH. In each temperature acclimation, the animals were given free access to either normal diet or high-fat diet. The compositions for the two diets were presented in Table I. Animals in any groups were housed individually throughout the experiment. Body mass was not significantly different among four groups at the start of the experiment

($F_{3,36}$=0.821, P>0.05).

Table I.- **Compositions of high- and low-fat diet on dry mass in *A. draco*.**

Contents	Low-fat diet (LFD)	High-fat diet (HFD)
Crude fat (%)	6.2	21.4
Crude protein (%)	20.8	17.6
Neutral detergent fiber (%)	21.5	19.6
Acid detergent fiber (%)	12.5	10.6
Ash (%)	10.0	8.5
Caloric value (kJ/g)	17.5	19.7

Measurement of metabolic rates

Metabolic rates were measured by using an AD ML870 open respirometer (AD Instruments, Australia) at 25°C within the thermal neutral zone (TNZ). Gas analysis was using a ML206 gas analysis instrument, the temperature was controlled by a SPX-300 artificial climatic incubator (±0.5°C). The metabolic chamber volume was 500ml with air flow of 200 ml/min. The *A. draco* were stabilized in the metabolic chamber for at least 60 min prior to the RMR measurement, oxygen consumption was recorded for more than 120 min at 1 min intervals. Ten stable consecutive lowest readings were taken to calculate RMR (Li and Wang, 2005). Method of metabolic rate calculation was described in detail by Hills (Hills, 1972). NST was induced by subcutaneous injection of norepinephrine (NE) (Shanghai Harvest Pharmaceutical Co. Ltd) and measured at 25°C. Two consecutive highest recordings of oxygen consumption in more than 60 min at each measurement were taken to calculate the NST (Zhu *et al*., 2010b). The doses of NE were approximately 0.8-1.0 mg/kg according to dose-dependent response curves that were carried out before the experiment and the equation described by Heldmaier (1971) : norepinephrine dosage (mg/kg)=$6.6M^{-0.458}$(g).

Food trials

Energy intake was measured from the balance of food intake and fecal output (Rosenmann and Morrison, 1974). Animals were individually housed in metabolic cages (20 cm × 15 cm × 15 cm) without nest materials for one week. They were fed excess quantity of food each day between 10:00 and 11:00 am. On the following day they were weighed and the remaining food, feces were collected. Residual food and feces were dried to constant weight and dry mass determined to the nearest 0.1 g. The energy contents of the samples were measured using an automatic bomb calorimeter (model YX-ZR/Q, Changsha, China). Gross energy intake (GEI), digestible energy intake (DEI) and digestibility of energy were calculated according to the literature (Drozdz, 1975).

GEI (kJ/day) = Dry food intake (g/day) × caloric value (kJ/g) of dry food;
DEI (kJ/day) = GEI-[mass of feces (g/d) × gross energy content of feces (kJ/g)];
Digestibility = DEI/GEI.

Measurements of enzyme activity, UCP1 content

Mitochondrial protein concentration was determined by the Folin phenol method (Lowry *et al*., 1951) with bovine serum album as standards. The COX activity was measured by the polarographic method using oxygen electrode units (Hansatech Instruments Ltd., Norfolk, England) (Sundin *et al*., 1987).

UCP1 content was measured by Western blotting. Total BAT protein (15 µg per lane) was separated in a discontinuous SDS-polyacrylamide gel (12.5% running gel and 3% stacking gel) and blotted to a nitrocellulose membrane (Hybond-C, Amersham). To check for the efficiency of protein transfer, gels and nitrocellulose membranes were stained after transferring with Coomassie brilliant blue and Ponceau red, respectively. Unspecific binding sites were saturated with 5% nonfat dry milk in PBS. UCP1 was detected using a polyclonal rabbit UCP1 (1:5000) as a primary antibody (Abcam, Shanghai) and peroxidase-conjugated goat anti-rabbit IgG (1:5000) (Jackson Immuno. Inc., USA) as the second antibody. Enhanced chemoluminescence (ECL, Amersham Biosciences, England) was used for detection of UCP signal. UCP1 concentration was determined from area readings by using Scion Image Software (Scion Corporation) and was expressed as relative units (RU) (Li and Wang, 2005).

Measurement of hormone concentration

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

The concentrations of triiodothyronine (T_3) and thyroxine (T_4) in serum were determined using RIA kits (China Institute of Atomic Energy). These kits were validated for all species tested by cross-activity. The intra- and inter-assay coefficients of variation were 2.4% and 8.8% for the T_3, 4.3% and 7.6% for T_4, respectively.

Morphology

The gastrointestinal tracts (stomach, small intestine, caecum and large intestine) were removed and weighed (± 1 mg) from animals of four groups (49 days). The stomach and intestines were then rinsed with saline to eliminate all gut contents before being dried and reweighed. The remaining carcass and all the organs were dried in an oven at 60 °C to constant mass (at least 72 h), and then weighed again to obtain the dry mass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus (Li and Wang, 2005).

Statistical analysis

Data were analyzed using the software package SPSS 15.0. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance using Kolmogorov-Smirnov and Levene tests, respectively. Changes of body mass over the whole acclimation was measured with Two-way ANOVA with repeated measurements (temperature × diet), followed by Tukey's HSD post-hoc to determine the daily variation. RMR, NST, energy parameters, serum leptin level, T_3, T_4 concentrations, COX activity, UCP1 level, and body composition were analyzed by a two-way ANOVA or two-way ANCOVA, and body mass or body carcass mass used as a covariate where appropriate. Post-hoc comparisons were conducted using the Tukey's HSD test when required. To detect possible associations of serum leptin level with body fat mass and energy intake, we used Pearson-correlation analysis. Results are presented as means ± SEM and $P < 0.05$ was considered to be statistically significant.

RESULTS

Body mass

Body mass (BM) of *A. draco* were affected significantly by temperature, but not by diet or the interaction of temperature and diet, BM by increased in WH group, while in CL group BM showed decreasing (temperature, $F_{1,36}$=9.568, P <0.01; diet, $F_{1,36}$=0.758, P>0.05; temperature×diet, $F_{1,36}$= 1.235, P>0.05; Two-way ANOVA with repeated measures, temperature×diet) (Fig. 1). For the final BM, it was higher in WL and WH groups, and lower in the CH and CL groups.

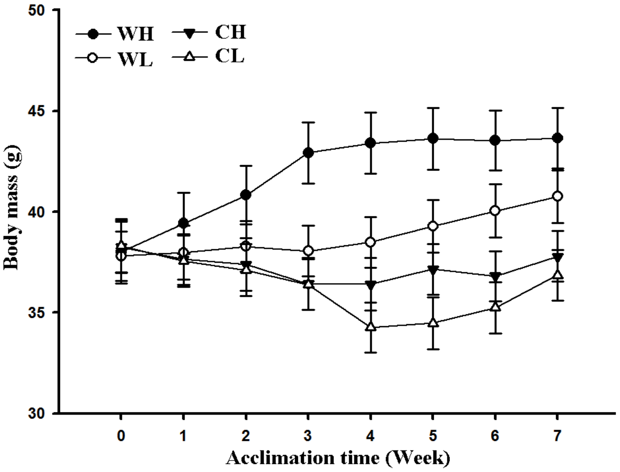

Fig. 1. Effect of cold exposure and high-fat diet on body mass in *A. draco*. WH, warm and high-fat diet; WL, warm and low-fat diet; CL, CH, cold and high-fat diet; cold and low-fat diet.

RMR and NST

Cold acclimation significantly increased RMR of *A. draco* (Fig. 2). Consistent with the changes of RMR, NST was also higher in cold-acclimated animals. Neither RMR nor NST were affected by diet or the interaction of temperature and diet (RMR, temperature, $F_{1,36}$=2.214, P <0.05; diet, $F_{1,36}$=1.021, P>0.05; NST, temperature, $F_{1,36}$=4.352, P<0.05; diet, $F_{1,36}$=0.542, P>0.05; Two-way

ANCOVA with body mass as a covariate, temperature×diet) (Fig. 2).

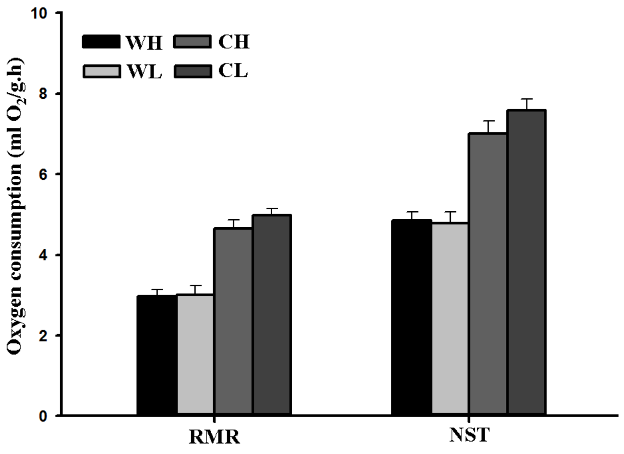

Fig. 2. Effect of cold exposure and high-fat diet on RMR and NST in *A. draco*. WH, warm and high-fat diet; WL, warm and low-fat diet; CL, CH, cold and high-fat diet; cold and low-fat diet.

GEI, DEI and digestibility

GEI was affected significantly by temperature and diet (temperature, $F_{1,36}$=6.362, $P < 0.01$; diet, $F_{1,36}$=15.369, $P<0.01$), which in CL and WL groups were higher than that in WH group. Similarly, DEI was affected significantly by temperature and diet and the interaction of temperature and diet (temperature, F=7.546, $P < 0.01$; diet, F=10.362, $P<0.01$; temperature×diet, F(df)= 3.214, $P<0.05$), which in CL and WL were higher than that in WH (Fig. 3A). Finally, digestibility was also affected significantly by temperature and diet and the interaction of temperature and diet (temperature, $F_{1,36}$=5.362, $P < 0.01$; diet, $F_{1,36}$=36.211, $P<0.01$; temperature×diet, $F_{1,36}$(try to make sure it is 1. it is related to the number of gourps = 9.652, $P<0.01$; Two-way ANCOVA with body mass as a covariate, temperature×diet), which in CH was higher than that in WL (Fig. 3B).

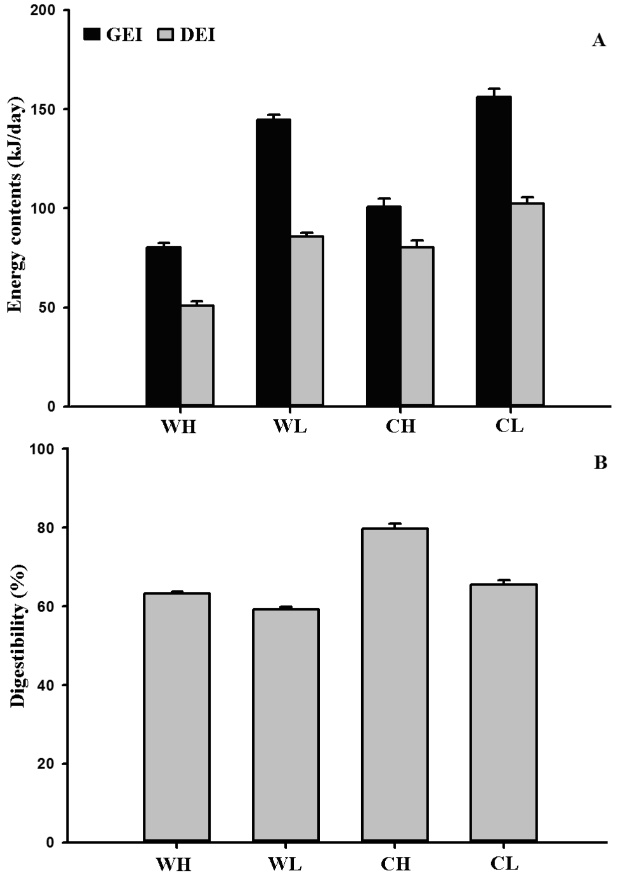

Fig. 3. Effects of cold exposure and high-fat diet on GEI, DEI (A) and digestibility (B) in *A. draco*. WH, warm and high-fat diet; WL, warm and low-fat diet; CL, CH, cold and high-fat diet; cold and low-fat diet.

Cytochrome c oxidase (COX) activity, UCP1 content and serum T_3, T_4 concentrations

Both Mt protein content and COX activity in liver were affected by temperature and the interaction of temperature and diet (Mt protein content, temperature, $F_{1,36}$=4.236, $P < 0.01$; temperature × diet, $F_{1,36}$=8.362, $P<0.01$; COX activity, temperature, $F_{1,36}$=5.368, $P < 0.01$; temperature × diet, $F_{1,36}$=11.236, $P<0.01$), which in CL group were higher than that in other groups of Mt protein content and COX activity (Table II). Mt protein content of BAT was affected by temperature and the interaction of temperature and diet (temperature, $F_{1,36}$=14.365, $P < 0.01$; temperature× diet, $F_{1,36}$=16.201, $P<0.01$), which in CL group were higher than that in other groups. In addition, BAT-COX activity was also affected by temperature and the interaction of temperature and diet (temperature, $F_{1,36}$=4.022, $P<0.05$; temperature× diet, $F_{1,36}$=8.624, $P<0.01$), which in CL group were higher than that in other groups. BAT-UCP1 contents was affected by

Table II.- **Effects of cold exposure and high-fat diet on COX activity, UCP1 content and T3, T4 concentrations in *A. draco*.**

Parameters	Warm		Cold	
	High-fat diet	Low-fat diet	High-fat diet	Low-fat diet
Liver				
Mt protein content (mg/g tissue)	25.36±0.62	23.58±0.36	23.24±0.35	30.31±0.55
COX activity (nmol/min g tissue)	123.02±6.54	89.36±5.36	91.36±6.05	168.69±8.32
BAT				
Mt protein content (mg/g tissue)	22.65±0.98	20.85±1.21	19.88±1.44	33.25±1.36
COX activity (nmol/min g tissue)	1421.02±23.65	1265.36±25.36	1255.58±25.84	1598±32.21
UCP1 content (RU)	1.06±0.05	0.98±0.03	1.27±0.04	1.10±0.10
Hormones				
T_4 (ng /ml)	68.81±3.47	70.89±5.36	66.36±10.32	68.32±8.36
T_3 (ng /ml)	2.01±0.11	1.98±0.09	2.06±0.12	4.36±0.16

temperature, which was notably higher in CL group of *A. draco* (temperature, $F_{1,36}$=4.523, P<0.05, Two-way ANCOVA, temperature × diet), which in CH group were higher than that in other groups. Finally, T_3 concentration was affected by temperature and the interaction of temperature and diet (temperature, $F_{1,36}$=6.521, P<0.05), which in CL group were higher than that in other groups, while there was no significant differences by temperature and diet on T_4 concentration.

Body compositions, body fat mass and serum leptin level

Body compositions and inner organs mass were not affected by diet. Dry carcass mass and body fat mass and serum leptin levels, however, were influenced significantly by temperature, which were higher in CH group than that in other groups of *A. draco* (carcass dry mass, $F_{1,36}$=10.653, P <0 01; body fat mass, $F_{1,36}$=5.245, P <0.01; serum leptin levels, $F_{1,36}$=3.072, P<0 05; Two-Way ANOVA, temperature × diet). In addition, temperature showed significant effects on wet mass of liver, lungs, spleen, and dry mass of liver and kidneys (liver wet mass, $F_{1,36}$=5.244, P<0.05; lungs wet mass, $F_{1,36}$=6.684, P<0.05; spleen wet mass, $F_{1,36}$=3.284, P<0.05; liver dry mass, $F_{1,36}$=3.895, P<0.05; kidney dry mass, $F_{1,36}$=4.984, P<0.05; Two-Way ANCOVA, temperature×diet, with body mass as a covariate), which were higher in CH and WH groups than that in other groups (Table III).

Stomach wet mass was significantly affected by diet, which was higher in WL and CL groups of *A. draco* ($F_{1,36}$=6.589, P<0.01). Diet showed significant effect on size and wet mass of small intestine (size, $F_{1,36}$=2.954, P<0.05; wet mass, $F_{1,36}$=6.322, P<0.01), which was higher in CL group. Simultaneously, dry mass of small intestine were affected by temperature ($F_{1,36}$=5.695, P<0.01), which was lower in WH. For large intestine, size, wet mass and dry mass were affected significantly by diet (size, $F_{1,36}$=3.265, P<0.05; wet mass, $F_{1,36}$=21.254, P<0.01; dry mass, $F_{1,36}$=4.258, P<0.05), which were lower in WL. In addition, wet mass and dry mass of large intestine were affected significantly by temperature (wet mass, $F_{1,36}$=5.236, P <0.05; dry mass, $F_{1,36}$=3.985, P<0.05). Temperature influenced significantly caecum wet mass ($F_{1,36}$=3.214, P<0.05; Two-Way ANCOVA, temperature×diet, with body mass as a covariate), which was higher in CL group (Table IV).

Similar to body fat mass, *A. draco* fed high-fat diet intended to show higher serum leptin level compared with the *A. draco* fed low-fat diet. Serum leptin level showed a positively correlated with body fat mass (r=0.397, P<0.05; Fig. 4), but no relationship correlated with gross energy intake.

DISCUSSION

In the present study, we measured the effect of temperature and diet on energy metabolism in *A. draco*, cold exposure could significantly increased energy intake, NST, COX activity and UCP1

Table III.- **Effects of cold exposure and high-fat diet on body compositions, body fat mass and serum leptin levels in *A. draco*.**

Parameters	Warm		Cold	
	High-fat diet	Low-fat diet	High-fat diet	Low-fat diet
Dry carcass mass (g)	18.36±1.02	14.36±0.98	12.21±1.35	11.85±1.13
Body fat mass (g)	5.37±0.12	4.54±0.06	4.40±0.08	4.25±0.07
Serum leptin levels (ng/ml)	1.97±0.09	1.81±0.07	1.70±0.06	1.65±0.07
Wet mass (g)				
Liver	0.214±0.121	0.192±0.082	0.203±0.090	0.172±0.112
Heart	0.236±0.012	0.219±0.062	0.213±0.065	0.218±0.014
Lung	0.273±0.033	0.251±0.035	0.213±0.029	0.249±0.039
Spleen	0.029±0.001	0.019±0.001	0.030±0.002	0.023±0.001
Kidneys	0.223±0.002	0.179±0.001	0.216±0.002	0.195±0.002
Dry mass (g)				
Liver	0.394±0.031	0.385±0.029	0.428±0.021	0.380±0.034
Heart	0.045±0.008	0.031±0.002	0.025±0.003	0.042±0.006
Lung	0.046±0.009	0.055±0.012	0.026±0.006	0.033±0.005
Spleen	0.0022±0.0002	0.0018±0.0004	0.0022±0.003	0.0181±0.0002
Kidneys	0.045±0.005	0.029±0.005	0.039±0.004	0.034±0.003

Table IV.- **Effects of cold exposure and high-fat diet on digestive tract morphology in *A. draco*.**

Parameters	Warm		Cold	
	High-fat diet	Low-fat diet	High-fat diet	Low-fat diet
Stomach				
Size (cm)	1.89±0.01	2.01±0.01	1.95±0.02	1.99±0.03
Wet mass (g)	0.38±0.03	0.44±0.02	0.36±0.02	0.46±0.03
Dry mass	0.083±0.001	0.096±0.02	0.095±0.09	0.09±0.05
Small intestine				
Size (cm)	43.32±1.31	42.85±0.98	41.86±1.02	45.65±0.86
Wet mass (g)	0.71±0.04	0.62±0.04	0.66±0.02	0.86±0.05
Dry mass	0.035±0.009	0.02±0.005	0.02±0.01	0.043±0.01
Large intestine				
Size (cm)	14.42±0.005	17.19±0.004	15.26±0.007	15.01±0.005
Wet mass (g)	0.34±0.015	0.50±0.02	0.36±0.02	0.54±0.03
Dry mass	0.04±0.001	0.04±0.001	0.02±0.001	0.04±0.001
Caecum				
Size (cm)	5.56±0.05	5.92±0.06	5.62±0.06	5.89±0.02
Wet mass (g)	0.45±0.022	0.51±0.02	0.39±0.03	0.61±0.02
Dry mass	0.05±0.001	0.04±0.001	0.05±0.001	0.05±0.001

content and serum T_3 concentrations; diet did not influence the heat production capacity significantly, but the high fat diet reduced energy intake, increase in digestibility significantly.

Energy intake and digestive tract morphology

Ambient temperature plays an important role in regulating physiological and behavioral responses in small mammals, which usually exhibit extraordinary physiological adaptations to extremely low temperatures in winter (McNab, 2002). Energy intake was increased during cold exposure in small mammals such as Brandt's voles and Mongolian gerbils (Li and Wang, 2005; Zhang and Wang, 2007a, b). In the present study, We found that GEI and DEI were increased under cold acclimation in *A. draco*. Food quality is also the main factor to influence energy intake and digestibility as well as energy balance in small mammals. In our study, GEI, DEI and digestibility were significantly affected by diet. *A. draco* fed on high fat diet reduced energy intake and digestion, and increased digestibility, similar to *O. curzoniae*, *Dicrostonyx groenlandicus* (Gross *et al*., 1985; Nagy and Negus, 1993; McNab, 2002).

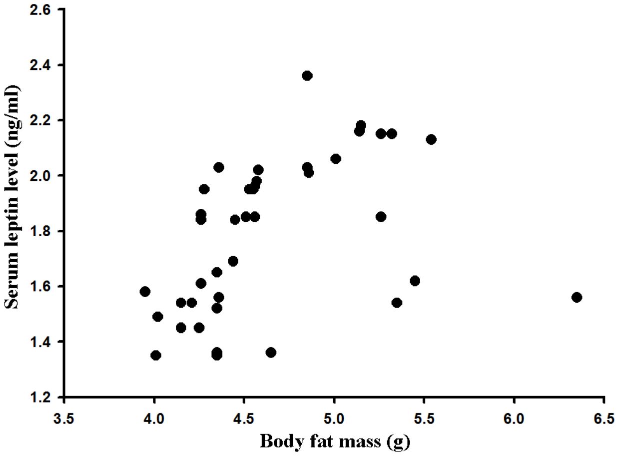

Fig. 4 Correlations between serum leptin levels and body fat mass in *A. draco* acclimated to cold exposure and high-fat diet.

Regulation of digestive tract morphology had the vital significance for animals' adaptation to changes in energy intake and digestibility (Derting and Bogue, 1993). In the present study, wet mass and dry mass of large intestine and small intestine were significantly affected by temperature and/or diet, CL group was higher than that of other groups in *A. draco* after 7 weeks acclimation. Many small mammals fed on low fat diet showed similar results in the digestive tract, such as *O. curzoniae* (Gross *et al*., 1985), rats (El-Harith *et al*., 1976), *Octodon degus* (Bozinovie *et al*., 1997) and *M. unguiculatus* (Pei *et al*., 2001). When facing with different food quality, *A. draco* showed plasticity in digestive tract morphology. Theoretically, if the gastrointestinal tract volume kept constant, high fat diet led to slow down the turnover rate of digestive tract, food retention time will be extended, thereby causing the increase of digestion rate. In the present study, *A. draco* fed on high fat diet probably maintain energy balance by reducing food turnover rate and increasing the absorption efficiency (Pei *et al*., 2001).

RMR, NST, COX activity, UCP1 level and serum T_3, T_4 concentrations

It is evident that many winter-active small mammals enhance BMR and NST for survival in the cold (Lovegrove, 2003), such as *Phodopus sungorus* (Heldmaier and Steinlechner, 1981). The increase in RMR were further supported by the biochemical markers examined in the present study, including higher MP content and COX activity in liver. Food is also one of the main factors affecting RMR (McNab, 1986). Many studies have confirmed that low quality diet may lead the lower RMR in mammals (Williams *et al*., 2004). In the present study, RMR was not affect by high-fat diet significantly, does not support hypothesis in Cork (1994): prediction of the direct relationship between the food and BMR in the evolutionary. The enhancement of NST for many seasonal small mammals is an important countermeasure adapted to low temperature environment (Jansky, 1973). In our study, cold temperature induced increase of NST in *A. draco*, but not affected by diet. BAT is the major site for this sympathetic-induced NST response (Ricquier and Bouillaud, 2000). In general, it is believed that the increase of COX activity and UCP1 content showed that enhancement of capacity on BAT heat production (Klingenspor *et al*., 2000). In the present study, it showed that BAT MP concentration was affected by temperature and diet, BAT-COX activity is regulated by temperature and interaction between temperature and diet, which in CL group were higher than that in other groups. The BAT UCP1 content was not affected by the diet, but increased significantly under low temperature, similar to changes of NST. The main function of thyroid hormones is to simulate thermogenesis, so the changes of the level of thyroid hormones may reflect the increased thermogenesis under cold conditions (Tomasi and Horwitz, 1987). T_3 concentration was affected by temperature and the interaction of temperature and diet, which in CL group were higher than that in other groups. Some studies showed similar results, such as in *Mesocricetus auratus* (Tomasi and Horwitz, 1987) and *Simodon hispidus* (Tomasi and Michell, 1994). But T_4 concentration was not affected significantly by temperature and diet, which may need further study.

Body mass, body fat mass and serum leptin level

Changes of body mass were closely related with the ability of adaptation for mammals under cold environment (Swanson, 2001). Body mass of mammals showed different changes under cold exposure, such as body mass of *D. groenlandicus* and *M. auratusz* increased body mass during cold acclimation (Nagy and Negus,1993; Jansky *et al*., 1986); body mass of *M. unguiculatus* and *Acomy cahirinus* did not change during cold acclimation (Günduz, 2002); body mass of *P. sungorus*, *E. miletus* and *A. chevrieri* decreased during cold acclimation (Klaus *et al*., 1988; Zhu *et al*., 2010a,b; 2011). Our study found that low temperature inhibited the growth of body mass in *A. draco*. Body mass decreased in the first 4 weeks, and then to maintain the relative stability, and slightly increased. However it showed high fat diet did not significantly affect body mass of *A. draco*, similar to *Psammomys obesus* (Degen *et al*., 2000). Consistent with changes of body mass, carcass dry weight, body fat mass were not affected by the food quality, and affected obviously by temperature in *A. draco*, body fat mass in WH group was significantly higher than that of cold groups. Body composition was very important to the maintenance of energy metabolism and regulation of body mass. To adapt to the environment of the seasonal fluctuations, mammals showed regulation of body mass as well as changes of body composition (Selman *et al*., 2001). In the present study, body compositions and inner organs mass were not affected by diet, however, were influenced significantly by temperature, and were higher in CH group of *A. draco*. It further confirmed that changes in body mass and body composition induced by temperature was a strategy adapt to the seasonal changing environment (Li and Wang, 2005).

Leptin, as an important endocrine regulation factor, plays an important role in energy metabolism, lipid metabolism, body mass regulation, the development of reproductive system and the immune and other physiological processes in mammals (Zhang *et al*., 1994). Serum leptin level was affected by environmental condition, such as temperature and photoperiod (Klingenspor *et al*., 1996, 2000). In the present study, serum leptin level was significantly affected by ambient temperature in *A. draco*, which in WH group was significantly higher than that of cold groups, but was not affected by food quality. Many studies suggested that role of leptin in energy metabolism and body mass regulation may be related to changes in environmental conditions (Mercer and Tups, 2003). In our study, we found that cold temperature inhibited growth in body mass, reduced body fat mass, what was not affected by high fat diet in *A. draco*, which indicated that *A. draco* can resist high fat diet induced obesity under cold temperature.

In conclusion, *A. draco* showed physiological regulations in body mass, thermogenesis and energy budgets under different temperature and food quality. Cold temperature enhanced thermogenesis. High-fat diet significantly increased the digestibility and body fat mass for *A. draco* exposed to warm temperature. *A. draco* showed lower body mass and body fat mass in response to cold temperature, which showed the resistance to high-fat diet-induced obesity (highlighted sentences conflict with each other, please check)

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