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Full Length Research Paper

Effects of photoperiod on energy metabolism and thermogenesis in the Melano-bellied oriental vole (*Eothenomys melanogaster*)

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Seasonal changes in photoperiod are important environmental factors for small mammals to adjust some physiological processes. In order to further understand the adaptive physiological strategies in Melano-bellied oriental vole (*Eothenomys melanogaster*) and integrated changes from organism to cell levels, the responses of this species were studied in different photoperiods. Experiment data indicated that Melano-bellied oriental vole exhibited adaptive physiological regulations when exposed to different photoperiod. The plasticity in energy intake, basal metabolic rate (BMR) and nonshivering thermogenesis (NST) was very important for the regulations in energy balance and thermogenesis in Melano-bellied oriental vole exposed to different photoperiod. Change in brown adipose tissue (BAT) cytochrome c oxidase (COX) activity and liver COX activity was one of the cellular basis for adaptive thermogenesis.

Key words: Melano-bellied oriental vole, photoperiod, energy metabolism, brown adipose tissue, cytochrome c oxidase, thermogenesis.

INTRODUCTION

Photoperiod is an environmental timer for its stability and predictability in all the year round. Photoperiod influences animal behaviour, molt, propagation and metabolize (Bartness et al., 2002; Barrett et al., 2005; Bowers et al., 2005). It also influences basal metabolic rate (BMR) and nonshivering thermogenesis (NST) of small mammals (Haim et al., 1999; Wang et al., 1999; Zhao and Wang, 2005). For example, *Sekeetamys calurus*, *Mus macedonicus* (Haim, 1996; Haim et al., 1999), *Meriones unguiculatus* (Zhao and Wang, 2006a) and *Microtus brandti* (Zhao and Wang, 2006b) increase their energy intake under short photoperiod, but *Dicrostonyx groenlandicus* brings down its energy intake (Powell et al., 2002). The change of animal fat mass is also an important factor that has effect on body weight. Body weight of

Phodopus sungorus reduces under short photoperiod mainly because of abasement of body fat mass (Wade and Bartness, 1984; Youngstrom and Bartness, 1998). Under short photoperiod and low temperature, many field mice such as *Dipodomys ordii*, *P. sungorus*, *Mesoerietus auratus*, their rest metabolic rate (RMR) and NST increased (Hou et al., 1995). They belong to photosensitivity animals. However, *Clethrionomys rutilus* only increase thermogenesis in cold environment and belong to unphotosensitivity animals (Heldmaier et al., 1989).

Melano-bellied oriental vole (*Eothenomys melanogaster*) is attached to (Rodentia) (Cricetidae) (Chinchilla). It belongs to especial class group of Microtinae. Melano-bellied oriental vole inhabits the Central China region of oriental realm, such as Zhejiang, Anhui, Fujian, Guangdong, Sichuan and so on (Zhuge, 1989). It is also distributed in India Assam, northern part of Burma and central south Peninsula. It inhabits grove, shrub, boscage, farmland, upland and so on (Ran et al., 1998; Zhao et al., 1994). Although some work has been done on the feeding habit, population ecology and digestive tract form in Melano-bellied

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Abbreviations: BMR, Basal metabolic rate; NST, nonshivering thermogenesis; BAT, brown adipose tissue; COX, cytochrome c oxidase.

Zhugue, 1986; Wang et al., 2007), there are no data available yet on energy metabolism and thermogenesis. Therefore, in the present study, we measured the body weight, body fat mass, energy intake, BMR, NST, mitochondrial protein content and cytochrome c oxidase activity of this species from Zhejiang, Yiwu and attempt to correlate them with their distribution patterns.

MATERIALS AND METHODS

Animals

Animals were live-trapped in the mountain regions (29°10'48" - 29°19'12"N, 120°2'24" - 120°3'36"E) of Yiwu City, Zhejiang Province, China. It belongs to subtropical monsoon climate. The mean annual temperature is 17.2°C, average monthly maximum temperature in July is 29.3°C, and average monthly minima temperature in January is 4.6°C. Animals were divided into two groups stochastically (SD group, 8L:16D; LD group, 16L:8D) and were domesticated three weeks, respectively. From the domestication experiment beginning, body weight was measured at two days intervals by electronic balance (FA1104, accurate to 0.1 g).

Body fat mass was measured by rope extraction method. Viscera of animals (retaining fat of digestive tract) were removed and parched in heating oven at 60°C. It was comminuted by small muller. 2 g sample was weighed and was put in 1/2 - 2/3 aether extraction bottle. It was heated in 70°C water bath to make aether circumfluence at 120 - 150 drip/h lasting 5 h. The formula used was:

$$\text{Percentage of body fat (\%)} = \frac{\text{Body fat mass (g)}}{\text{Dry carcass mass (g)}} \times 100\%$$

Energy intake trials

Energy intake was measured by metabolizable cage. Feeding was done at definite time with definite quantity and body weight was weighed. With two days intervals, remnant food and dejecta were collected and heated in heating oven at 60 weighing. Remnant food and dejecta were separated. Some samples were weighed (between 0.5 and 1.0 g, accurate to 0.0001 g). Thermal value was measured by Hunan microcomputer calorimeter. According to Drozd (1975), Grodzinski and Wunder (1975)'s proposal, urine energy was calculated by 2% of digested energy. Other parameters were calculated according to formula as follows:

$$\text{Energy intake (kJ/d)} = \text{Dry matter intake (g/d)} \times \text{Food thermal value (kJ/g)}$$

$$\text{Dejecta energy (kJ/d)} = \text{Dejecta dry weight (g/d)} \times \text{Dejecta thermal value (kJ/g)}$$

$$\text{Digested energy (kJ/d)} = \text{Energy intake (kJ/d)} - \text{Dejecta energy (kJ/d)}$$

$$\text{Metabolizable energy intake (kJ/d)} = \text{Digested energy (kJ/d)} - \text{Urine energy (kJ/d)} = \text{Digested energy (kJ/d)} \times 98\%$$

$$\text{Digestibility (\%)} = \frac{\text{Digested energy (kJ/d)}}{\text{Energy intake (kJ/d)}} \times 100\%$$

$$\text{Efficiency of metabolizable energy intake (\%)} = \frac{\text{Metabolizable energy intake (kJ/d)}}{\text{Energy intake (kJ/d)}} \times 100\%$$

were measured. BMR was measured using the closed-circuit respirometer at 25°C (±0.5°C) (TNZ) according to Górecki (1975). Briefly, the metabolic chamber size was 3.6 L, and the chamber temperature was controlled within ±0.5°C by water bath. Carbon dioxide and water in the metabolic chamber were absorbed with KOH and silica gel. Subjects were weighted before and after each test. Animals fasted 4 hours prior to being put into the metabolic chamber. After 60 min stabilization in the chamber, metabolic measurement was conducted for 60 min. Oxygen consumption was recorded at 5 min intervals. Two continuous stable minimum recordings were taken to calculate BMR. Maximum NST was defined as the maximum metabolic response to norepinephrine (NE) and was induced by a subcutaneous injection of NE at 25°C. The mass-dependent dosage of NE (Shanghai Harvest Pharmaceutical Co. LTD) was calculated according to Heldmaier (1971): NE dosage (mg/kg) = 6.6Mb - 0.458 (g). Two continuous stable maximal recordings were used to calculate maximum NST. Oxygen consumption reached peak values within 15 and 30 min after NE injection. BMR and NST were expressed as ml O₂ · h⁻¹ and corrected to standard temperature and air pressure (STP) conditions.

Sample collection and isolation of mitochondria

One day after metabolic measurement, the animals were killed by decapitation. The liver and muscle were quickly removed, placed in ice-cold sucrose-buffered medium, cleaned of any adhering tissue, blotted and weighed, followed by homogenization for the isolation of mitochondria (1:15, w/v) with medium A (containing 250 mM sucrose, 10 mM TES, 1 mM EDTA, 64 AM BSA, pH 7.2) (Cannon and Lindberg, 1979). The brown adipose tissue (BAT) was weighed and followed by homogenization for the isolation of mitochondria (1:15, w/v) with medium A (containing 250 mM sucrose, 10 mM TES, 1 mM EDTA, 64 μM BSA, pH 7.2) (Cannon and Lindberg, 1979). The supernatant was then centrifuged at 8740×g for 10 min at 4°C, and the resulting pellet was resuspended (1:1, w/v) with ice-cold medium B (containing 100 mM KCl, 20 mM TES, 1 mM EGTA, pH 7.2). The protein content of mitochondria was determined by the Folin phenol method with bovine serum albumin as standard (Lowry et al., 1951).

Measurements of COX activity

The activities of cytochrome c oxidase (COX) in liver and BAT were measured polarographically at 30°C using a Clark electrode. The respiration medium contained 100 mM KCl, 20 mM TES, 1 mM EDTA, 2 mM MgCl₂, 4 mM KH₂PO₄, 60 mM BSA, pH 7.2. Ten ml aliquot taken from the supernatant and 30 ml cytochrome c (37.9 mg/ml) were added to the electrode and the activity of COX was measured in a final volume of 2 ml (Sundin et al., 1987). The activity of COX was expressed as n mol O₂ (min. mg mitochondrial protein)⁻¹ (Wiesinger et al., 1989).

Statistics

Data were analyzed using the SPSS package (version 12.0 for windows). All data was checked up by variance homogeneity in accordance with normal distribution. Body weight, percentage of body fat, energy intake, digested energy and so on were checked up by independent sample t-test between SD and LD. Effect of

photoperiod on body weight, energy intake, digested energy and so on were checked up by least significant difference. BMR, NST, mitochondrial protein content and COX activity were checked up by 9000 Afr. J. Biotechnol.

independent sample t-test between SD and LD. To exclude effect of body weight, initial data was regulated by power of 0.67 body weight

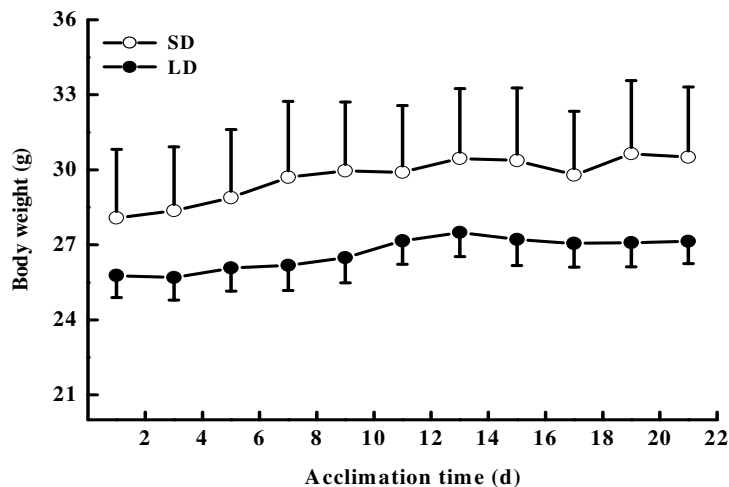


Figure 1. The changes in body weight of Melano-bellied oriental vole exposed to short photoperiod (SD, 8L:16D) and long photoperiod (LD, 16L:8D).

Table 1. The effects of photoperiod on body composition in Melano-bellied oriental vole.

Parameter	SD	LD	p value
Sample size (n)	10	10	
Initial Body mass (g)	28.4±2.7	25.9±0.9	0.395
Final Body mass (g)	30.7±2.9	27.1±1.0	0.257
Wet carcass mass (g)	20.43±1.95	15.92±0.56	0.039
Dry carcass mass (g)	10.69±0.98	8.02±0.25	0.017
Body fat mass (g)	2.86±0.34	1.09±0.05	0.001
Percentage of body fat (%)	26.42±1.23	13.71±0.71	0.001
Fat-free dry mass (g)	7.83±0.67	6.93±0.24	0.222

SD: Short photoperiod (8L:16D); LD: Long photoperiod (16L:8D). Values are expressed as mean ± standard error of mean.

(Hayssen and Lacy, 1985). All values were presented as mean ± SE in the text and $P < 0.05$ was taken as statistically significant.

RESULTS

Change of body weight

There was no significant differences in body weight between SD group and LD group from the beginning ($t = 0.872$, $df = 18$, $P > 0.05$) (Figure 1). During the whole domestication, no significant effects were found in body weight between SD group and LD group (SD, $LSD > 0.05$; LD, $LSD > 0.05$, $t = 3.572$, $df = 218$) (Figure 1). At the end of the experiment, no significant effects were found between groups ($t = 1.170$, $df = 18$, $P > 0.05$) (Figure 1).

Percentage of body fat

Body fat mass and percentage of body fat of SD group were significantly higher than LD group ($t = 5.142$, $df = 18$, $P = 0.001$; $t = 8.942$, $df = 18$, and $P = 0.001$). Wet carcass mass and dry carcass mass also had significant differences between groups ($t = 2.232$, $df = 18$, $P = 0.039$; $t = 2.633$, $df = 18$, and $P = 0.017$). However, fat-free dry mass had no significant differences between groups ($t = 1.264$, $df = 18$, $P = 0.222$) (Table 1).

Energy intake and digestion

Significant effects were found in dry matter intake (corrected with 0.67 power of body mass) and energy intake (corrected with 0.67 power of body mass) between SD group and LD group ($t = 5.079$, $df = 218$, $P < 0.001$; $t =$

5.079, $df = 218$, $P < 0.001$). No significant effects were found in digested energy (g/g 0.67.d), metabolizable energy intake (g/g 0.67.d), digestibility and efficiency of metabolizable energy intake, along with domestication was prolonged, there were significant differences in dry

matter intake, energy intake, digested energy, metabolizable energy intake, digestibility and efficiency of metabolizable energy intake on SD group (LSD, $P < 0.05$, $t = 24.861$, $df = 49$; $t = 24.861$, $df = 49$; $t = 23.924$, $df = 49$; $t = 23.924$, $df = 49$; $t = 73.157$, $df = 49$) (Table 2). There were significant differences in dry matter intake and energy intake on LD group (LSD, $P < 0.05$, $t = 76.985$, $df = 49$) (Table 2). No significant effects were found in digested energy, metabolizable energy intake, digestibility and efficiency of metabolizable energy intake (LSD, $P > 0.05$, $t = 36.452$, $df = 49$; $t = 36.452$, $df = 49$; $t = 93.508$, $df = 49$; $t = 93.508$, and $df = 49$) (Table 2). Therefore, photoperiod had significant effects in energy metabolism in Melano-bellied oriental vole. Along with domestication was prolonged, energy metabolism took place in mutation.

Changes of BMR and NST

There was no significant difference in BMR and NST between SD group and LD group in initial experiment ($t = -0.609$, $df = 18$, $P > 0.05$ for BMR; $t = -0.302$, $df = 18$, $P > 0.05$ for NST). After three weeks, BMR of SD group was significantly higher than BMR of LD group ($F = 2.040$, $df = 18$, and $P = 0.012$) and significant differences were found in NST between SD group and LD group ($F = 1.655$, $df = 18$, and $P = 0.018$). In final experiment, significant differences were not found in relatively increasing oxygen consumption (NST - BMR)/BMR between SD group and LD group. However, significant differences were found in absolutely increasing oxygen consumption (NST - BMR) (Table 3).

COX activity of BAT and liver

After three weeks, no significant effects were found in BAT mass, liver mass and so on between SD group and LD group in Melano-bellied oriental vole. COX activity of BAT and liver in SD group were significantly higher than COX activity of BAT and liver in LD group ($F = 3.041$, $df = 18$, and $P < 0.001$; $F = 0.669$, $df = 18$, and $P < 0.001$) (Table 4).

DISCUSSION

Body weight and fat

The change of photoperiod was an important environmental factor to induce seasonal variations in body weight of many mammals (Li and Wang, 2005a; Li and Wang,

2005b; Bartness and Wade, 1985; Gottreich et al., 2000). Energy metabolism has differential response to photoperiod, which leads to effect of photoperiod on body weight that has species difference (Wade and Bartness, 1984).

Short photoperiod alone could induce body weight increment in *D. groenlandicus* (Maier and Feist, 1991). It
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could as well induce body weight significant abasement in *P. sungorus* (Claude et al., 1998). Effect of photoperiod on body weight also had seasonal difference and sexual difference (Hou et al., 1995) such as, body weight of *D. groenlandicus* declined in summer and significantly increased in winter (Maier and Feist, 1991). It was related to age of animals too. For example, growing development of juvenile was inhibited in *P. sungorus* (Adam et al., 2000) and *M. brandti* (Liu and Fang, 2001) and adolescent growth was delayed. Furthermore, effect of photoperiod on body weight was related to victualage and active law too (Heideman and Bronson, 1990). Melano-bellied oriental vole was nonhibernating rodent. No effect was detected in body mass by 3-week photoperiod acclimation.

Body fat mass is important for maintaining body weight. In order to adapt to seasonal undulation, some animals makes adaptive adjustment in body weight, body fat mass and organ mass (Voltura and Wunder, 1998; Selman et al., 2001), such as, *M. brandti* and *M. unguiculatus* (Li and Wang, 2005a,b). The change of body weight is related to body fat mass, body weight of *P. sungorus* comes down accordingly as body fat mass decline under short photoperiod (Klingenspor et al., 1996). Under breeding season and low temperature, *M. brandti* brings down body weight and consumes body fat mass (Liu et al., 2003; Song and Wang, 2001). After 3-week photoperiod acclimation, no effect was detected in body mass in Melano-bellied oriental vole. It increases energy outgo to maintain body fat mass under short photoperiod. That explains short photoperiod inducing energy intake increment to maintain large body weight and body fat mass.

Golley (1962) put forward that body fat mass changed according to age and season. Substance of photoperiod is seasonal signal under field conditions. Voltura and Wunder (1998) thought that when resources were scarce, animals had to use body fat mass to resist intimidation of environment. Taking Melano-bellied oriental vole as an example, short photoperiod group showed increases body fat mass and percentage of body fat, compared to long photoperiod group. Long photoperiod corresponds to summer, Melano-bellied oriental vole has enough food and does not need to reserve body fat mass in summer. As short photoperiod corresponds to winter, quantity of food goes down, animal needs to store body fat mass in winter. Furthermore, increment of body fat mass is favorable for heat insulation and heat preservation. It is important for Melano-bellied oriental vole to decrease temperature and metabolic rate.

Energy metabolism

Effect of photoperiod was detected in energy intake. Energy intake has different response to photoperiod, such as energy intake increases in *M. macedonicus* (Haim et al., 1999) and *S. calurus* (Haim, 1996). No effect of photoperiod was detected in energy intake in *L. brandtii* and *M.*

Table 2. Effects of photoperiod on energy intake and assimilation in Melano-bellied oriental vole (*Eothenomys melanogaster*).

Parameters	Acclimation period (day)				
	1	3	9	15	21
Short photoperiod sample size	10	10	10	10	10
Body weight (g)1	28.2280±1.8870 ^a	28.2240±1.8260 ^a	29.4270±1.8852 ^a	30.1390±1.9202 ^a	30.2200±1.8898 ^a
Dry matter intake					
(g/d)	5.2700±0.6629 ^a	6.7370±0.3824 ^{ab}	6.8560±0.5304 ^b	6.9300±0.3269 ^b	5.2040±0.3235 ^{ac}
(g/g 0.67.d)	1.7006±0.0024 ^a	1.8705±0.0011 ^b	1.8770±0.0015 ^b	1.8837±0.0010 ^b	1.7098±0.0010 ^a
Energy intake					
(kJ/d)	85.8905±10.8032 ^a	109.7996±6.2325 ^{ab}	111.7391±8.6440 ^b	112.9451±5.3271 ^b	84.8148±5.2724 ^{ac}
(kJ/ g0.67.d)	27.7170±0.0392 ^a	30.4862±0.0184 ^b	30.5914±0.0239 ^b	30.7009±0.0178 ^b	27.8670±0.0155 ^a
Digested energy					
(kJ/d)	68.6178±10.0675 ^a	97.3723±5.4935 ^b	94.8730±6.5859 ^{bc}	102.0376±5.1555 ^b	75.7859±4.8158 ^{ac}
(kJ/ g 0.67.d)	13.4244±0.0478 ^a	17.1797±0.0128 ^b	16.3886±0.0253 ^b	18.4244±0.0258 ^b	16.2616±0.0220 ^b
Metabolizable energy intake					
(kJ/d)	67.2455±9.8661 ^a	95.4249±5.3836 ^b	92.9756±6.4542 ^{bc}	99.9968±5.0524 ^b	74.2702±4.7194 ^{ac}
(kJ/ g 0.67.d)	13.1560±0.0468 ^a	16.8361±0.0125 ^b	16.0608±0.0248 ^b	18.0559±0.0252 ^b	15.9363±0.0215 ^b
Digestibility (%)	78.18±0.12 ^a	88.78±0.03 ^b	85.60±0.08 ^b	90.68±0.04 ^b	89.71±0.04 ^b
Efficiency of metabolizable energy intake (%)	76.61±0.12 ^a	87.01±0.03 ^b	83.88±0.08 ^b	88.87±0.04 ^b	87.92±0.04 ^b
Long photoperiod Sample size	10	10	10	10	10
Body weight (g)1	25.8255±0.5940 ^a	25.7330±0.6115 ^a	26.2825±0.6681 ^a	27.1940±0.6834 ^a	27.1005±0.6337 ^a
Dry matter intake					
(g/d)	5.7190±0.6742 ^a	5.4920±0.4568 ^a	5.9710±0.2992 ^a	5.7820±0.3459 ^a	4.1060±0.2088 ^b
(g/g 0.67.d)	1.7497±0.0024 ^a	1.7430±0.0015 ^a	1.7989±0.0012 ^a	1.7798±0.0014 ^a	1.5874±0.0010 ^b
Energy intake					
(kJ/d)	93.2083±10.988 ^a	89.5086±7.4450 ^a	97.3154±4.8757 ^a	94.2350±5.6372 ^a	66.9196±3.4023 ^b
(kJ/ g 0.67.d)	28.5164±0.0386 ^a	28.4073±0.0239 ^a	29.3189±0.0153 ^a	29.0072±0.0224 ^a	25.8715±0.0123 ^b
Digested energy					
(kJ/d)	81.1370±11.875 ^a	81.4471±7.7085 ^a	89.7446±5.0182 ^a	85.0099±5.5804 ^a	59.5591±3.9875 ^b
(kJ/ g 0.67.d)	15.5883±0.0517 ^{ab}	16.9258±0.0309 ^{ab}	18.0272±0.0210 ^a	16.8456±0.0254 ^{ab}	14.8221±0.0287 ^b
Metabolizable energy intake					
(kJ/d)	79.5143±11.637 ^a	79.8182±7.5543 ^a	87.9497±4.9179 ^a	83.3097±5.4688 ^a	58.3680±3.9077 ^b
(kJ/ g 0.67.d)	15.2765±0.0507 ^{ab}	16.5873±0.0303 ^{ab}	17.6667±0.0206 ^a	16.5087±0.0249 ^{ab}	14.5256±0.0281 ^b
Digestibility (%)	84.12±0.12 ^a	90.32±0.05 ^b	91.98±0.04 ^b	89.94±0.04 ^b	88.91±0.05 ^{ab}
Efficiency of metabolizable energy intake (%)	82.43±0.12 ^a	88.51±0.05 ^b	90.14±0.04 ^b	88.14±0.04 ^b	87.13±0.05 ^{ab}

Data are presented as mean±SE, the different superscripts in the same row indicates significant differences.

Table 3. The effects of photoperiod on BMR and NST in Melano-bellied oriental vole.

Parameter	SD	LD	p value
Sample size	10	10	
Initial basal metabolic rate (BMR)	7.12±0.09	7.32±0.05	0.550
Final basal metabolic rate (BMR)	9.50±0.22	7.33±0.11	0.012
Initial nonshivering thermogenesis (NST)	21.95±0.38	22.34±0.17	0.766
Final nonshivering thermogenesis (NST)	29.29±0.73	22.84±0.28	0.018
Final absolutely increasing oxygen consumption (NST - BMR)	19.80±0.55	15.51±0.17	0.029
Final relatively increasing oxygen consumption (NST - BMR)/BMR(%)	2.09±0.04	2.13±0.02	0.748

SD: Short photoperiod (8L:16D); LD: Long photoperiod (16L:8D). Values are expressed as mean ± standard error of mean.

Table 4. The effects of photoperiod on mitochondrial (Mt) protein content and COX activity of BAT and liver in Melano-bellied oriental vole.

Parameter	SD	LD	p value
Sample size	10	10	
Brown adipose tissue (BAT)			
mass (g)	0.029±0.008	0.028±0.004	ns
Mitochondrial protein content (mg/g tissue)	10.550±1.0364	11.865±1.825	ns
Cytochrome c oxidase activity (n mol O ₂ /min.mg protein)	128.507±2.911	109.560±1.837	<0.001
Liver			
Mass (g)	0.907±0.201	1.168±0.056	ns
Mitochondrial protein content (mg/g tissue)	13.769±2.365	15.004±2.476	ns
Cytochrome c oxidase activity (n mol O ₂ /min.mg protein)	31.831±0.683	22.579±0.900	<0.001

SD: Short photoperiod (8L:16D); LD: Long photoperiod (16L:8D). Values are expressed as mean ± standard error of mean.

unguiculatus (Li et al., 2003). Effect of photoperiod in energy intake has species difference and it may rest with animal ecology and animal geography origin (Haim and Shabtay, 1998). It may relate to habitat, *Saccostomus campestris* is more sensitive when compared to ilk which inhibits in zone of rainfall (Ellison et al., 1992). Ellison et al. (1992) thought this difference was related to energy exploitability and climate badness. Effect of photoperiod was also detected in energy outgo. Under short photoperiod, energy outgo of *D. groenlandicus* reduces and body weight increases. Energy outgo of *P. sungorus* increases, food intake and body weight reduces as well (Rousseau et al., 2002). This study showed that Melano-bellied oriental vole exposed to short photoperiod, increased in energy intake. Effect of photoperiod on energy metabolism has species specificity. Food is not abundant in winter and climate is cold. Animals need to store energy. Short photoperiod alone could increase energy intake in Melano-bellied oriental vole. Photoperiod is an important cue to induce seasonal variations in energy intake in Melano-bellied oriental vole.

Energy metabolism and body weight

Body weight is a mark that reflects nutrition status. The necessity of maintaining a favorable body weight depends on the balance of energy acquisition and energy expenditure. Energy intake decrease, energy outgo increase, digestibility and efficiency of metabolizable energy intake decline, active quantity increase and active rhythm change induce body weight decline (Knopper and Boily, 2000; Larkin et al., 1991). Body weight and energy metabolism have different response to photoperiod, such as, short photoperiod induce body weight decline in *P. sungorus* with energy intake decline and energy outgo increase (Rousseau et al., 2002; Heldmaier, 1989; Mercer et al., 1997, 2000; Klingenspor et al., 2000). Short photoperiod induces body weight increase in *D. groenlandicus* (Powell et al., 2002). Energy intake does not increase and energy outgo declines (Nagy and Negus, 1993; Krol et al., 2005). Larkin et al. (1991) thought that the effect of photoperiod on body weight in *Rattus norvegicus* was not related to energy intake. Larkin et al.

weight under short photoperiod was lower compared to long photoperiod in *R. norvegicus* because energy was distributed in *R. norvegicus* under long photoperiod.

Melano-bellied oriental vole exposed to short photoperiod showed increase in energy intake and immobile in body weight, increase in energy intake is used in thermogenesis and energy outgo increase. Thus, maintain energy balance and body weight stably.

BMR and NST

Photoperiod becomes environmental timer for its stability and predictability and influences BMR and NST of small mammals (Haim et al., 1999; Wang et al., 1999; Zhao and Wang, 2005). Photoperiod is one of important cues to induce BMR of many small mammals. Such as *Acomys russatus* (Haim and Zisapel, 1995), *M. macedonicus* (Haim et al., 1999) and *M. guentheri* (Banin et al., 1994). The researches about effects of temperature, photoperiod and so on in BMR were reported. Low temperature could induce BMR increase for some animals. However, short photoperiod could induce BMR increase for some animals. Effects of different environmental factors in BMR for small mammals were not the same and had interspecies differences (Wang et al., 1999). Wang et al. (1999) thought that no effect was detected in BMR in *Ochotona curzoniae* under short photoperiod, cold temperature could heighten BMR. Compared to *O. curzoniae*, short photoperiod could induce BMR increase in *M. oeconomus*. Larkin et al. (1991) found that BMR declined in *R. norvegicus* under short photoperiod. It was thought that photoperiod influenced animals of ingestive mode and active rhythm, animals lowered BMR in order to decrease energy expenditure under short photoperiod.

It has been found that small mammals showed seasonal changes in thermogenesis. Thermogenesis capability of *Dipus sagitta*, *M. meridianus*, *Phodopus roborovskii*, *Cricetulus barabensis* (Bao et al., 2002), *Acomys russatus*, *Acomys cahirinus* (Kronfeld-Schor et al., 2000) and *Glaucomys volans* (Merritt et al., 2001) increased in winter, especially NST. There was sensibility about the effects of photoperiod in thermogenesis for animals. No significant effects were found in *Clethrionomys glareolus*, *Clethrionomys rutilus* under short photoperiod (Feist and Feist, 1986; Heldmaier et al., 1989). However, many field mice such as *A. cahirinus* (Haim and Zisapel, 1999), *M. unguiculatus* (Li and Wang, 2005) and so on were regulated in thermogenesis. Short photoperiod in autumn could regulate adaptive thermogenesis increase in *M. brandti* (Li et al., 1995), no significant effects were found in summer (Hou et al., 1995). Variations about animal heat and day rhythm in NST were found in the same specie, two populations of *Apodemus mystacinus* in thermogenesis under short photoperiod (Spiegel and Haim, 2004). Short photoperiod alone could induce NST increase in *P. sungorus*

study showed that Melano-bellied oriental vole exposed to short photoperiod in BMR, NST absolutely increasing oxygen consumption were significantly higher than Melano-bellied oriental vole exposed to long photoperiod. It showed that photoperiod alone significantly influenced BMR and NST in Melano-bellied oriental vole. Short photoperiod alone could increase thermogenic capacity in Melano-bellied oriental vole. Photoperiod is an important cue to induce seasonal variations in thermogenic capacity in Melano-bellied oriental vole.

Cytochrome oxidase (COX) activity of BAT and liver

Brown adipose tissue (BAT) is the main site for NST. Factors that influenced and controlled BAT could also induce changes in NST. It has been found that mitochondrial adaptive changes in BAT were the biochemistry basis of NST capability increase (Feist, 1984; Jansky, 1973). For different voles in different habitats, there was different response for BAT mitochondrial protein when it was exposed to cold temperature and different thermogenesis (Rafael et al., 1985; Oufara et al., 1988; Wang et al., 1996). It has been found that enhanced thermogenic capacity in BAT is associated with increased cytochrome c oxidase (COX) activity of BAT (Wang et al., 1999; Rafael and Vsiansky, 1985). Huang et al. (2006) showed that short photoperiod could induce increased RMR and NST by means of increased COX activity of BAT and liver in *S. dauricus*. This study showed that Melano-bellied oriental vole exposed to short photoperiod could increase NST and COX activity of BAT. There was sensibility for effects of photoperiod in COX activity of BAT in Melano-bellied oriental vole.

In thermal regulation for small mammals, some organs and tissues such as liver, heart and muscle have played a role besides BAT owing to total protein of these organs and tissues, increased mitochondrial protein or COX activity in mitochondrial. It has been found that *Gerbillinae* in desert zone showed stable mitochondrial protein in muscle and decreased enzyme activity exposed to low temperature (Oufara et al., 1988). Significant effect was detected in total protein of these organs and tissues such as liver, heart, muscle, mitochondrial protein content and enzyme activity during cold domestication in *M. oeconomus*. It showed that they indeed participated in thermal regulation (Wang et al., 1996). Liver was thought to be an important energy expenditure organ with contribution to BMR (Selman et al., 2001). Compared to long photoperiod, Melano-bellied oriental vole exposed to short photoperiod showed increases in COX activity in liver. It was similar with changes of BMR and NST. It showed that changes of BMR and NST under photoperiod were related to mitochondrial respiration in liver. The regulation of COX activity in liver may be one potential mechanism that photoperiod could induce changes of

BMR and NST.

In short, no effect was detected in body mass by 3-week photoperiod acclimation in Melano-bellied oriental vole.

Compared to long photoperiod, Melano-bellied oriental vole exposed to short photoperiod showed increase in energy intake, body fat mass and percentage of body fat.

Photoperiod is one of important cues to induce seasonal variations in energy balance and body fat in Melano-bellied oriental vole. Melano-bellied oriental vole exposed to short photoperiod showed increase in BMR, NST, COX activity in liver and BAT. There were significant differences between short photoperiod group and long photoperiod group from the level of individual to the level of cell. Short photoperiod alone could increase thermogenic capacity.

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