



Effects of level of food restriction on body mass and thermogenesis in a tree shrew (*Tupaia belangeri*)

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ARTICLE INFO

Received 13 Sept 2018
Revised 28 Nov 2018
Accepted 26 Dec 2018
Available online 30 Dec 2018

Keywords: *Tupaia belangeri*; thermogenesis; cytochrome c oxidase

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ABSTRACT

This study investigates the energy strategies of a small mammal in response to food shortages as a function of level of food restriction (FR). We subjected tree shrews (*Tupaia belangeri*) to different levels of FR and measured body mass, survival rate, resting metabolic rate (RMR), nonshivering thermogenesis (NST) and cytochrome c oxidase (COX) activity of brown adipose tissue (BAT). Body mass, RMR, NST and COX activity of BAT were significantly decreased in FR animals. After 4 weeks of FR, survival rates ranged from 80% in animals restricted to 90% of *ad libitum* food intake to 30% in those restricted to 60% of *ad libitum* intake. These results indicate that tree shrews, known for their relatively high metabolic rates, are sensitive to periods of FR, which supports the metabolic switch hypothesis.

INTRODUCTION

The level of energy metabolism of wildlife is affected by many environmental and physiological factors. Food is one of the most important factors. The quality and quantity of food in nature will be affected by uneven spatial distribution, seasonal change or drastic environmental change, which will lead wildlife to face food in some periods of their life cycle, such as lack of resources and starvation (Hammond and Diamond, 1997). Changes in food resources affect the body weight, heat production, growth, reproduction and other physiological activities of small mammals (Bacigalupo and Bozinovic, 2002). According to the metabolic switch hypothesis, an ability to adjust metabolic rate plays a key role in animals adapted to periods of food shortage, enabling them to "switch down" their resting metabolic rate to survive these shortages (Merkt and Taylor 1994). The adaptive strategies adopted by animals in response to food shortages are also affected by other environmental conditions and their own behavior. In a 50% food restriction (FR) experiment, the golden thorn rat hair (*Acomys russatus*), a non-hoarding species, survived more than 6 weeks, while the foxtail gerbil (*Gerbillus dasyurus*), a hoarding species, survived only 2 weeks. These data suggest that hoarding species may have a lower tolerance to food shortages than non-hoarding species (Gutman et al., 2006). Whether this is generally true, or whether the survival of hoarding species during food shortages also depends on their capacity for metabolic rate adjustment, remains unclear.

The tree shrew *Tupaia belangeri* (Mammalia: Scandentia: Tupaiidae) is a small mammal native to Southeast Asia. It is widely distributed throughout Southern China, India and Southeast Asia in farmland and shrub habitat. It has been reported that tree shrews show seasonal thermogenesis (Zhu et al., 2012) and have a higher resting metabolic rate (RMR) in their thermoneutral zone (TNZ) than other small mammals (Xiao et al., 2010). The aim of this study was to test the metabolic switch hypothesis in tree shrews by investigating their energy strategies in response to food shortages as a function of level of FR. We subjected tree shrews to different levels of FR and measured changes in body mass, metabolic thermogenesis and cytochrome c oxidase (COX) activity of brown adipose tissue (BAT).

MATERIALS AND METHODS

Animals and experimental designs

Tree shrews, *T. belangeri*, were wild-captured from farmland and shrub near Luquan County (25°26'–25°62' N, 102°13'–102°57' E, altitude 1650–1700 m) in Yunnan Province, China. The area is located in the northern part of the Yunnan Plateau and has a subtropical plateau climate. The geological landform is complex with large variations in surface relief. The temperature shows marked changes with altitude, and while the annual temperature changes are small, there are large daily temperature fluctuations. Tree shrews were transported to the Animal Feeding Room of Yunnan Normal University, and individually housed in wire cages

(40×30×30 cm). Animals were kept in a room maintained at 25 ± 1 °C with a natural photoperiod for 4 weeks prior to the experiment, and provided *ad libitum* food and water. The food contained the following ingredients (by weight): 5% milk, 5% sugar, and 90% cornmeal. All procedures were licensed under the Animal Care and Use Committee of the School of Life Science, Yunnan Normal University (approval ref. 13-0901-011).

We have randomly divided 50 adult tree shrews of similar body mass into five groups (N = 10 per group): controls (fed *ad libitum* for 4 weeks), FR-90%, FR-80%, FR-70% and FR-60% (restricted to 90%, 80%, 70% or 60%, respectively, of initial *ad libitum* food intake for 4 weeks). Survival rate (%) was calculated daily as number of surviving animals/10 × 100%. Body mass was recorded every two days, and metabolic thermogenesis and COX activity of BAT were measured at the end of the experiment.

Measurement of metabolic rates

Metabolic rates were measured using an AD ML870 open respirometer at 30 ± 0.5 °C within the TNZ as described previously (the TNZ of *T. belangeri* is 30–35 °C, Wang et al., 1994). The volume of the metabolic chamber was 760 ml and the temperature in the chamber was maintained within 0.5 °C by a SPX-300 temperature-controlled cabinet. The air flow was 200 ml/min, and a ML206 gas analyzer was used for gas analysis. Tree shrews were fasted for 3 h before being transferred into the metabolic chamber. After 1 h of adaptation to the chamber, metabolic measurements were conducted for another 1 h, during which oxygen consumption was read at 10 s intervals. RMR was calculated using two continuous, stable, minimum recordings (Wang et al., 1994) using the method of Hill (1972). Nonshivering thermogenesis (NST) was induced by a subcutaneous injection of norepinephrine (Shanghai Harvest Pharmaceutical) and measured at 30 °C. The dose of norepinephrine administered was approximately 0.8–1.0 mg/kg body mass, used based on dose-dependent response curves generated before the experiment (Zhu et al., 2008). NST was calculated using the two highest consecutive recordings of oxygen consumption (Wang et al., 1995).

Measurement of protein content of mitochondria and enzyme activity

Interscapular BAT was removed and weighed immediately after the experiment. Mitochondrial protein (MP) was prepared as described in a previous report (Wiesinger et al. 1989). MP content was determined by the Folin phenol method (Lowry et al. 1951), with bovine serum albumin as the standard. The COX activity of BAT was measured polarographically with oxygen electrode units (Hansatech Instruments, England; Sundin et al. 1987).

Statistical analysis

Data were analyzed using SPSS 15.0 software package. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov-Smirnov and Levene tests

,respectively. Body mass, RMR, NST and COX activity were analyzed by one-way analysis of variance (ANOVA) and significant group differences were further evaluated by Tukey post hoc test. Results were presented as mean ± SEM, and P < 0.05 was considered to be statistically significant.

RESULTS

FR resulted in the death of some animals. After 4 weeks of FR, the survival rate was 30% for FR-60%, 60% for FR-70%, and 80% for FR-80% and FR-90% (Fig. 1). There was no significant difference in body mass before the experiment among the groups (F4,45 = 0.028, P > 0.05, Fig. 2). After 4 weeks of FR, the body mass of FR-90%, FR-80%, FR-70% and FR-60% was decreased by 12.32%, 18.78%, 21.41% and 22.28%, respectively (FR-90%, F15,105 = 11.133, P < 0.01; FR-80%, F15,90 = 21.431, P < 0.01; FR-70%, F15,75 = 24.356, P < 0.01; FR-60%, F15,30 = 36.412, P < 0.01, Fig. 2). By FR day 7, there was a significant difference in body mass among groups (F4,42 = 3.074, P < 0.05). By FR day 28, the body mass of FR-80%, FR-70% and FR-60% was significantly lower than those of the controls and FR-90% (F4,29 = 20.015, P < 0.01, Fig. 2). The rate of change in body mass across the 4-week FR period was also significantly different among the groups (F4,29 = 9.762, P < 0.01, Fig. 2).

Food intake of control group was 12.36±1.25 g/d. FR-90%, FR-80%, FR-70% and FR-60% were fed 11.12±1.31, 9.89±0.97, 8.65±0.89 and 7.42±0.80 g/d, respectively. There was a significant difference in food intake among groups (F4,45 = 3.417, P < 0.05). FR had a significant effect on RMR (F4,28 = 6.441, P < 0.01) and NST (F4,28 = 6.814, P < 0.01). The RMR of FR-80%, FR-70% and FR-60% was significantly lower than that of the controls, and the NST of FR-70% and FR-60% was significantly lower than those of the controls and FR-90% (Fig. 4).

FR had significant effects on BAT mass (F4,28 = 3.346, P < 0.05, Fig. 4A) and COX activity of BAT (F4,29 = 4.116, P < 0.05, Fig. 4C), but had no significant effect on MP content (F4,29 = 1.174, P > 0.05, Fig. 4B). The BAT mass of FR-70% and FR-60% was significantly lower than that of the controls. The COX activity of FR-70% and FR-60% was significantly lower

than those of the controls and FR-90%.

DISCUSSION

Small mammals living in temperate regions often adapted to winter by reducing body mass (Voltura and Wunder, 1998). Seasonal food-deficient environments are conducive to reducing the absolute demand for energy (Nagy et al., 1995). In the present study of tree shrews, a 4-week period of FR resulted in mortality rates ranging from 20% in FR-90% to 70% in FR-60%. Gutman et al. (2006) suggests that non-hoarding species have higher tolerance to FR than hoarding species. As a hoarding species, the low tolerance of tree shrews to FR reported in our study is consistent with this hypothesis. It is unclear why tree shrews show such a low tolerance to food shortages. Species characterized by higher levels of metabolism are typically more sensitive to periods of FR (Song and Wang, 2003). According to the “mouse-to-elephant” curve, small animals have higher weight-specific RMRs than do big animals (Kleiber, 1932). Animals with higher metabolic rates require more energy and consume more frequent meals to fuel energy expenditure, rendering them more sensitive to periods of FR (Gutman et al., 2006, 2007). Compared with other small mammals, tree shrews are small and have high metabolic rates (Xiao et al., 2010). According to the metabolic switch hypothesis, animals respond to FR with a “switch down” of their RMR for survival (Merkt and Taylor, 1994). Many studies have shown that FR significantly reduces metabolic rates to establish a new energy balance, which supports the metabolic switch hypothesis (McCarter and Palmer 1992; Hambly et al. 2007; Zhao and Cao 2009). The tree shrews in our study also reduced their metabolic rate in response to FR. After 3 weeks of FR, the body mass of FR-90%, FR-80% and FR-70% was relatively stable, suggesting that they attained a new energy balance, which supports the metabolic switch hypothesis. Conversely, the body mass of FR-60% showed continued decline. In this FR-60% group, we suggest that the reduction in energy expenditure elicited by reducing their metabolic rate was not sufficient to compensate for the large degree of FR, resulting in continual loss of body mass and sometimes in death.

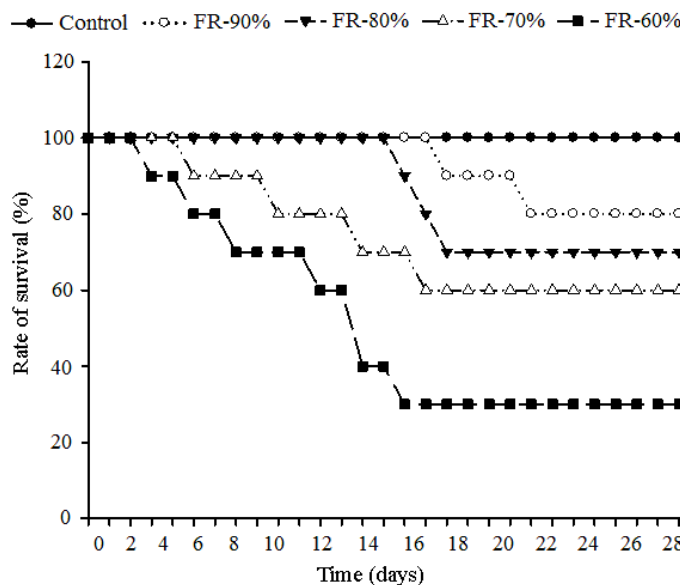


Fig. 1: The rate of survival in *Tupaia belangeri* during the course of food restriction.

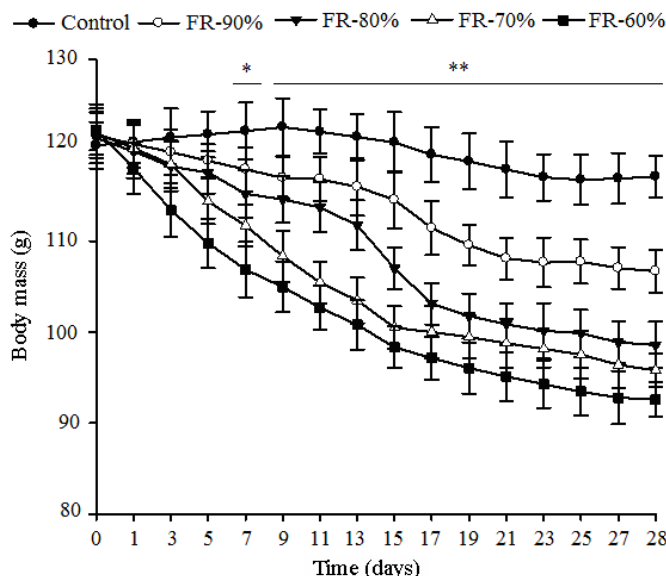


Fig. 2: Body mass in *Tupaia belangeri* during the course of food restriction. Different superscripts in each row indicate significant difference (P<0.05).

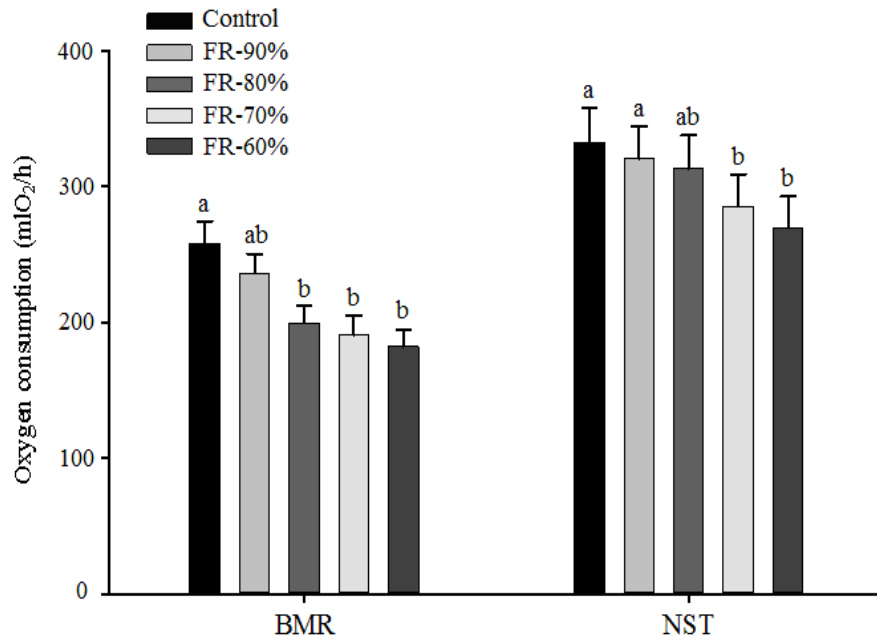


Fig. 3: RMR (A) and NST (B) in *Tupaia belangeri* during the course of food restriction. Different superscripts in each row indicate significant difference ($P < 0.05$).

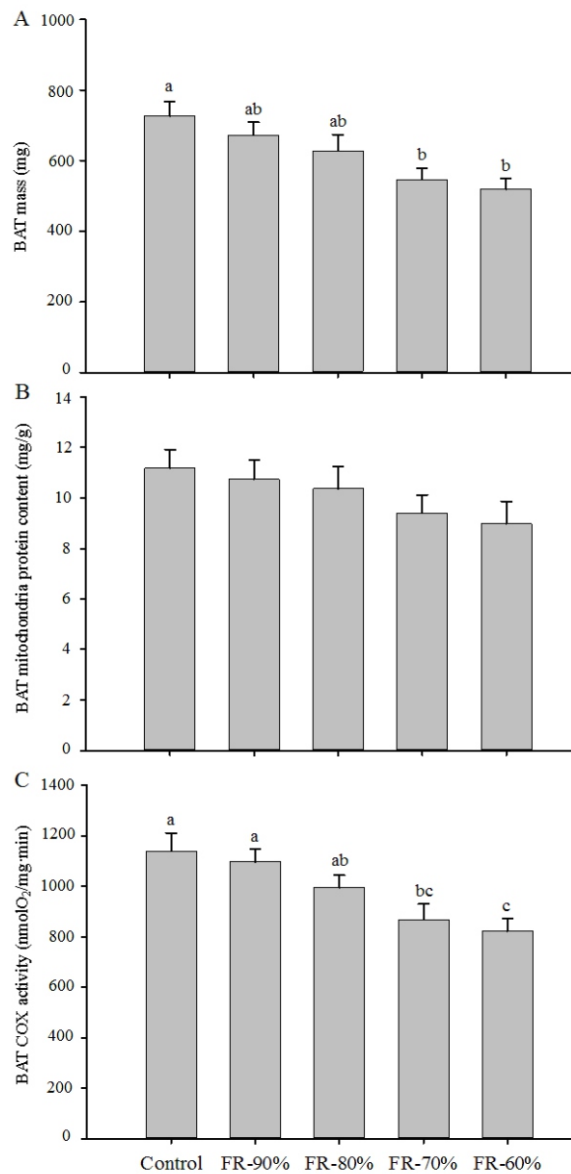


Fig. 4: BAT mass (A), protein content of mitochondria (B) and COX activity (C) in *Tupaia belangeri* during the course of food restriction. Different superscripts in each row indicate significant difference ($P < 0.05$).

CONCLUSION

In conclusion, survival rates ranged from 80% in animals restricted to 90% of ad libitum food intake to 30% in those restricted to 60% of ad libitum intake after 4 weeks of FR, the body mass of FR-80%, FR-70% and FR-60% was significantly lower than those of the controls and FR-90% by FR day 28. RMR, NST, BAT mass and COX activity of BAT were significantly decreased in FR animals compared with that of control group. These results indicate that for tree shrews, unlike other rodents, tolerance to a food shortage is affected by the degree of FR. Tree shrews have relatively high metabolic rates, which may render them less tolerant to food shortages.

Acknowledgement

This research was financially supported by National Science Foundation of China (No. 31760118; 31560126).

Disclosure statement: No potential conflict of interest was reported by the author.

Financial and proprietary interest: Nil

Financial support: National Science Foundation of China (No. 31760118; 31560126).

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