Octa Journal of Biosciences
International peer-reviewed journal
Octa J. Biosci. Vol. 6(1):09-12

ISSN 2321 – 3663
Jan-June 2018

Octa Journal of Biosciences

journal homepage: www.sciencebeingjournal.com

Scientific Planet Society
SPS

Effect of different food restriction levels on body mass regulation in *Apodemus chevrieri*

Gong Xue-na, Wan-Long Zhu

Key Laboratory of Ecological Adaptive Evolution and Conservation on Animals-Plants in Southwest Mountain Ecosystem of Yunnan Province Higher Institutes College, School of Life Science of Yunnan Normal University, Kunming; 650500, China

ARTICLE INFO

Received 05 March 2018
Revised 20 May 2018
Accepted 10 June 2018
Available online 30 June 2018

Keywords: *Apodemus chevrieri*; Body mass; Food restriction

Email: zwl_8307@163.com

ABSTRACT

To investigate the relationship between the energy strategy in response to different food restriction levels, body mass, resting metabolic rate (RMR), nonshivering thermogenesis (NST) and cytochrome c oxidase (COX) activity were measured in *Apodemus chevrieri*. The results showed that RMR, NST and COX activity were significantly decreased in restricted to 90% of ad libitum food intake, but survival rate was 40% in *A. chevrieri* restricted to 60% of ad libitum food intake. All of the results suggests that *A. chevrieri* decreased BMR under food restriction, providing a support for the “metabolism switch hypothesis”.

INTRODUCTION

Adaptive regulation of energy metabolism is important for animals to cope with changes in the natural environment. In the natural environment, many animals are faced with seasonal changes in food resources (Veloso and Bozinovic 1993). The energy strategy for food quantity changes in non hibernating small mammals are roughly divided into two categories: one is the reduction of energy metabolism in a food shortage environment, such as MF1 *Mus musculus* (Hambly and Speakman 2005), *Peromyscus maniculatus* (Gutman et al. 2007); the other is that the level of energy expenditure remains unchanged and even increased under food restriction, such as *KM Mus musculus* and *Cavia porcellus* (Williams et al. 2002; Zhao et al. 2009). “Metabolism switch hypothesis” pointed that the key to whether animals can adapt to changes in food resources is whether they have the ability to regulate metabolic rates, under the condition of limiting food intake, it is possible to adapt to the chronic food shortage environment only by changing the metabolic rate and decreasing the metabolic levels (Merkt and Taylor 1994). Research showed that with the food storage habits of *Gerbillus dasyurus* after being restricted to 50% of ad libitum food intake, which can only survive for 2 weeks, but under the same food restriction, *Acomys russatus* that do not have food storage habits can live for at least 6 weeks or even longer (Gutman et al. 2006). Therefore, it is assumed that animals with food storage habits have lower tolerance to food restriction.

Apodemus chevrieri is a inherent species in Hengduan mountain region, which had food storage behavior in winter. Previous studies had demonstrated the presence of a seasonal variation in body mass, thermogenesis and digestive tract morphology in A. chevrieri (Zhu et al. 2012). Random food deprivation decreased body fat mass and increased activity significantly (Zhu et al. 2016). It can be seen that the change of food quantity may play an important role in the evolutionary adaptation of thermogenesis in A. chevrieri. On the basis of the above studies, body mass, RMR, NST and COX activity in *A. chevrieri* with different levels of food restriction were measured. We hypothesize that *A. chevrieri* will change their thermogenesis and body mass to cope with different levels of food restriction.

MATERIALS AND METHODS

Animals and experimental designs

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. All animal procedures were licensed under the Animal Care and Use Committee of School of Life Sciences, Yunnan Normal University (Permit No.: 13-0901-011). 50 adult weight-matched *A. chevrieri* were housed individually (were maintained at 12L: 12D (light on at 08:00am), 25±1ºC, respectively), and kept for at least 2 weeks to familiarize with the environment. After the acclimatizing period, the animals were randomly assigned to the following five groups: control group (provided food ad libitum), 90% of ad libitum food intake (FR-90%), 80% of ad libitum food intake (FR-80%), 70% of ad libitum food intake (FR-70%), 60% of ad libitum food intake (FR-60%), each group was 10 samples. Animals were acclimated for 4 weeks. Food intake was calculated as the mass of food missing from the hopper, subtracting orts mixed in the bedding. Survival rate were recorded everyday. On day 0, body mass, resting metabolic rate (RMR), nonshivering thermogenesis (NST) were measured, on day 28, body mass, RMR, NST, and cytochrome c oxidase (COX) activity of brown adipose tissue (BAT) were also measured

Measurement of food intake

Each animal was put in a metabolic cage (20×15×15cm3) with no nest materials, and fed laboratory mice chow pellets. Animals were fed a fixed quantity at a set time(9.5–10.5g, 11:00 am), and the next day body mass was assessed, and residual food collected. Residual food was dried in a vacuum dryer until the mass was invariable.

Measurement of metabolic rates

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

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

Measurement of protein content of mitochondria and enzyme activity

BAT were carefully and quickly removed and weighted (0.1mg), and their adhering tissues separated. The organs were blotted, weighed, and placed in ice-cold sucrose-buffered medium and then homogenized for the isolation of mitochondria (Cannon and Lindberg 1979). The protein content of mitochondria was determined by the Folin phenol method with bovine serum albumin as standard (Lowry et al. 1951). The COX (EC 1.9.3.1) activity of BAT was measured with polarographic method using oxygen electrode (Hansatech Instruments LTD., 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

Food restriction causes some animals to die, 90% of *A. chevrieri* survived for 4 weeks after being restricted to 90% of ad libitum food intake, but survival rate was 40% in A. *chevrieri* restricted to 60% of ad libitum food intake (Fig. 1). Body mass before the experiment showed no significant differences among five groups (F4,45=0.69, $P>0.05$). On day 28, FR groups decreased body mass significantly compared with that of control groups (F4,31=9.36, $P<0.01$, Fig. 2). A. *chevrieri* showed no variations in RMR and NST before the experiment (RMR: F4,45= 0.45, $P>0.05$; NST: F4,45= 0.71, $P>0.05$). On day 28, food restriction decreased RMR and NST significantly in FR-80%, FR-70% and FR-60% groups compared with that of control groups (RMR: F4,31= 4.36, $P<0.01$, Fig. 3A; NST: F4,31= 6.98, $P<0.01$, Fig. 3B). Food restriction affected BAT mass significantly (F4,31= 4.23, $P<0.01$, Fig. 4A), the protein content of mitochondria showed no significant differences among five groups (F4,31= 1.24, $P>0.05$, Fig. 4B), but COX activity in BAT were found remarkable differences among five groups, which was lowest in FR-60% group (F4,31= 6.39, $P>0.05$, Fig. 4C).

DISCUSSION

The change of food resources has an important influence on the energy metabolism and survival of animals, and the tolerance of animals to food resource shortage may be related to whether they have food storage habits (Gutman et al. 2006). In the present study, the death of A. *chevrieri* occurred in different degrees of food restriction, mortality rate was 20% in FR-90% group, and mortality rate reached 60% in FR-60% group. This result is different from that of other rodents, such as *Gerbillus dasyurus* after being restricted to 50% of ad libitum food intake, which can survive for 2 weeks, and *Acomys russatus* can live for at least 6 weeks (Gutman et al. 2006). MF1 mice under FR-80% acclimation, rats under 90% and 60% FR-80% acclimation could survive for a long time without death (Hambly and Speakman, 2005). These results suggested that the adaptive ability of different animals to food restriction varies greatly and may be species specific. Gutman et al. (2006) predicted that animals with food storage habits exhibited less tolerance to food restriction relative to those without food storage habits. A. *chevrieri* has seasonal food storage habit, and showed low tolerance to food restriction, which is consistent with the prediction that small mammals showing food shortage behavior may have less ability to cope with decreases in food availability.

The reason why A. *chevrieri* exhibit low tolerance to food restriction is uncertain. At the interspecific level, the researchers compared the metabolic levels of A. *chevrieri* with other rodents, it was found that A. *chevrieri* had smaller size and higher metabolic level (Zhu et al. 2008). A large number of studies showed that higher metabolic rate means higher demand for energy, so they need to increase the frequencies of food intake to supplement the metabolic energy expenditure, compared with the larger animals, the metabolic rate is higher, more easily influenced by food shortages, higher metabolic rate in smaller mammals were more likely to be affected by a shortage of food resources. “Metabolism switch hypothesis” pointed that in the context of changing food resources, if animals have the ability to regulate metabolic rates, they can adapt to a chronic food shortage by reducing metabolic levels (Merkt and Taylor 1994). Many studies have found that food shortage leads to a significant reduction in metabolism, which were consistent with the hypothesis's predictions (Zhao et al. 2012). In the present study, food restriction decreased RMR and NST significantly on day 28, providing a support for the “metabolism switch hypothesis”. Moreover, tolerance of food limitation in A. *chevrieri* may associate with food restriction degree, although energy expenditure was reduced by reducing metabolic rates, which is insufficient to compensate for the decrease in energy intake due to food restriction, resulting in continued body mass loss and even death. The A. *chevrieri* has a relative higher metabolic rate, possibly because of its low tolerance to food restriction.

CONCLUSION

In conclusion, 90% of A. *chevrieri* survived for 4 weeks after being restricted to 90% of ad libitum food intake, but survival rate was 40% in A. *chevrieri* restricted to 60% of ad libitum food intake. On day 28, food restriction decreased RMR and NST significantly in FR-80%, FR-70% and FR-60% groups compared with that of control groups. Food restriction affected BAT mass significantly, the protein content of mitochondria showed no significant differences among five groups, but COX activity in BAT were found remarkable differences among five groups, which was lowest in FR-60% group, providing a support for the “metabolism switch hypothesis”.

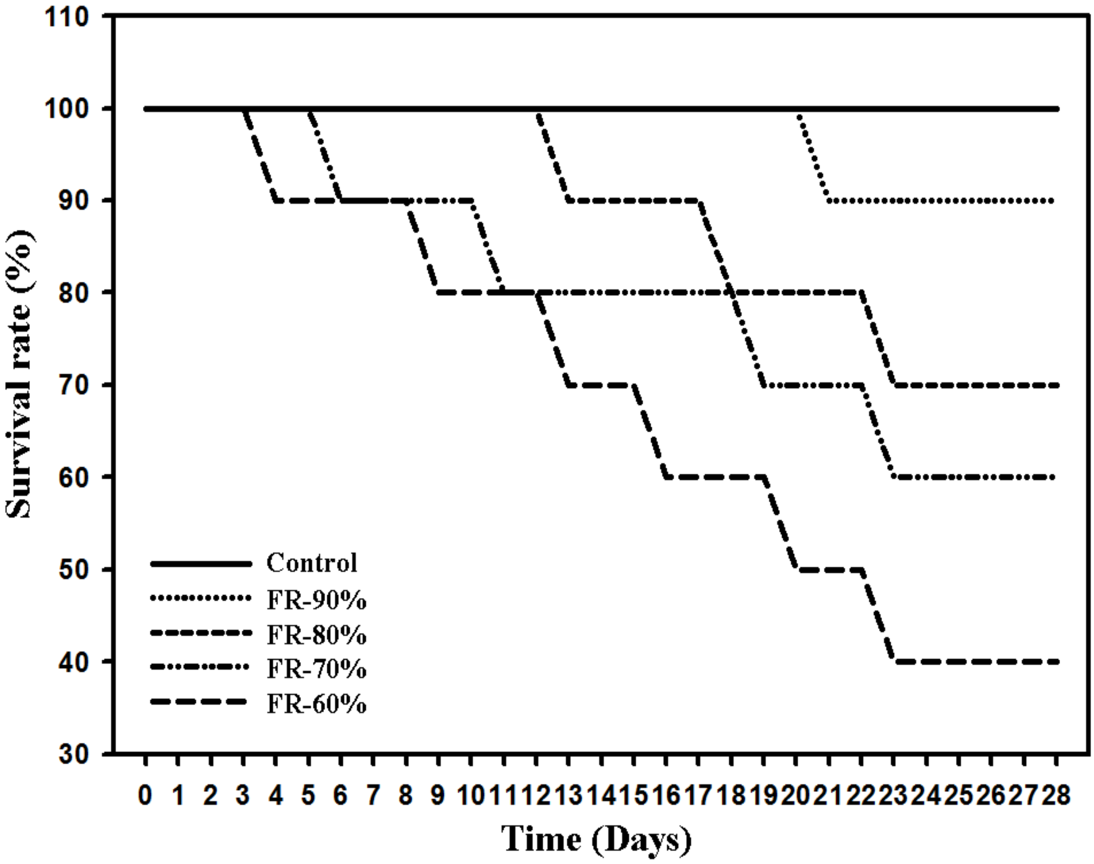

Figure 1: The rate of survival in *Apodemus chevrieri* during the course of food restriction

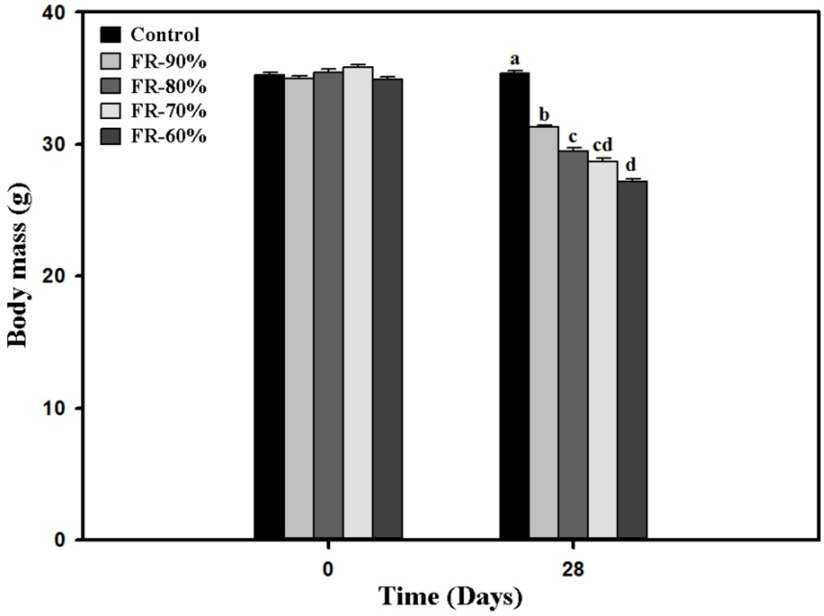

Figure 2: Body mass in *Apodemus chevrieri* during the course of food restriction. Different superscripts in each row indicate significant difference (P<0.05).

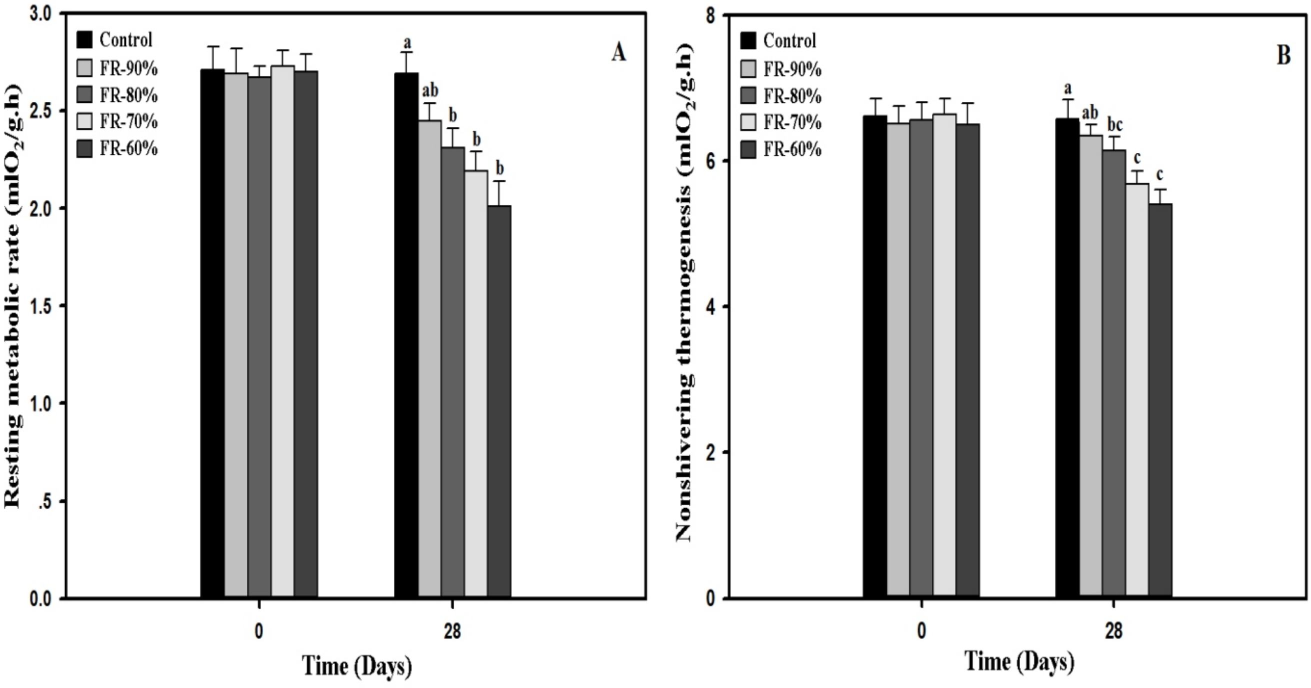

Figure 3: RMR (A) and NST (B) in *Apodemus chevrieri* during the course of food restriction. Different superscripts in each row indicate significant difference (P<0.05).

Acknowledgments

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).

REFERENCES

1. Cannon, B., Lindberg, U (1979); Mitochondria from brown adipose tissue: isolation and properties. Methods in Enzymology 55: 65–78.
2. Hambly, C., Speakman, J.R (2005); Contribution of different mechanisms to compensation for energy restriction in the mouse. Obesity Research 13: 1548–1557.
3. Gutman, R., Choshniak, I., Kronfeld-Schor, N (2006) Defending body mass during food restriction in Acomys russatus: a desert rodent that does not store food. American Journal of Physiology 290: R881–891.
4. Gutman, R., Yosha, D., Choshniak, I., Kronfeld-Schor, N (2007); Two strategies for coping with food shortage in desert golden spiny mice. Physiology & Behavior 90: 95–102.
5. Heldmaier, G (1971); Nonshivering thermogenesis and body size in 567 mammals. Journal of Comparative Physiology 73: B222–248.
6. Hill, R.W (1972); Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. Journal of Applied Physiology 33:261–263.
7. Li, X.S., Wang, D.H (2005) Regulation of body weight and thermogenesis in seasonally acclimatized Brandt's voles (Microtus brandti). Hormones and Behavior 48: 321–328.Merkt, J. and Taylor, C.R. 1994. A metabolic switch for desert survival. Proceedings of the National Academy of Sciences of the united states of America 91: 12313–12316.
8. Lowry, O.H., Rosbrough, N.J., Farr, A.L., Randall, R.J (1951); Protein measurement with the Folin phenol reagents. Journal of Biological Chemistry 193: 265–275.
9. Sundin, U., Moore, G., Nedergaard, J., Cannon, B (1987); Thermogenin amount and activity in hamster brown fat mitochondria: effect of cold acclimation. American Journal of Physiology 252: R822–832.
10. Veloso, C., Bozinovic, F (1993); Dietary and digestive constraints on basal energy metabolism in a small herbivorous rodent. Ecology 74: 2003–2010.
11. Williams, T.D., Chambers, J.B., Henderson, R.P (2002); Cardiovascular responses to caloric restriction and thermoneutrality in C57BL/6J mice. American Journal of Physiology 282: R1459–1467.
12. Zhao, Z.J., Wang, R.R., Cao, J., Pei, L.Y (2009); Effect of random food deprivation and refeeding on energy budget and development in mice. Zoological Research 30: 534–538.
13. Zhao, Z.J (2012); Effect of food restriction on energy metabolism and thermogenesis in striped hamster (Cricetulus barabensis). Acta Theriologica Sinica 32: 297–305.
14. Zhu, W.L., Jia, T., Lian, X., Wang, Z.K (2008); Evaporative water loss and energy metabolism in two small mammals, voles(Eothenomys miletus) and mice(Apodemus chevrieri) in Hengduan mountains region. Journal of Thermal Biology 33: 324–331.
15. Zhu, W.L., Yang, S.C., Zhang, L., Wang, Z.K (2012); Seasonal variations of body mass, thermogenesis and digestive tract morphology in Apodemus chevrieri in Hengduan mountain region. Animal Biology 62: 463–478.
16. Zhu, W.L., Wang, Z.K (2016); Effect of random food deprivation and re-feeding on energy metabolism, behavior and hypothalamic neuropeptide expression in Apodemus chevrieri. Comparative Biochemistry and Physiology, 201: A71–78.

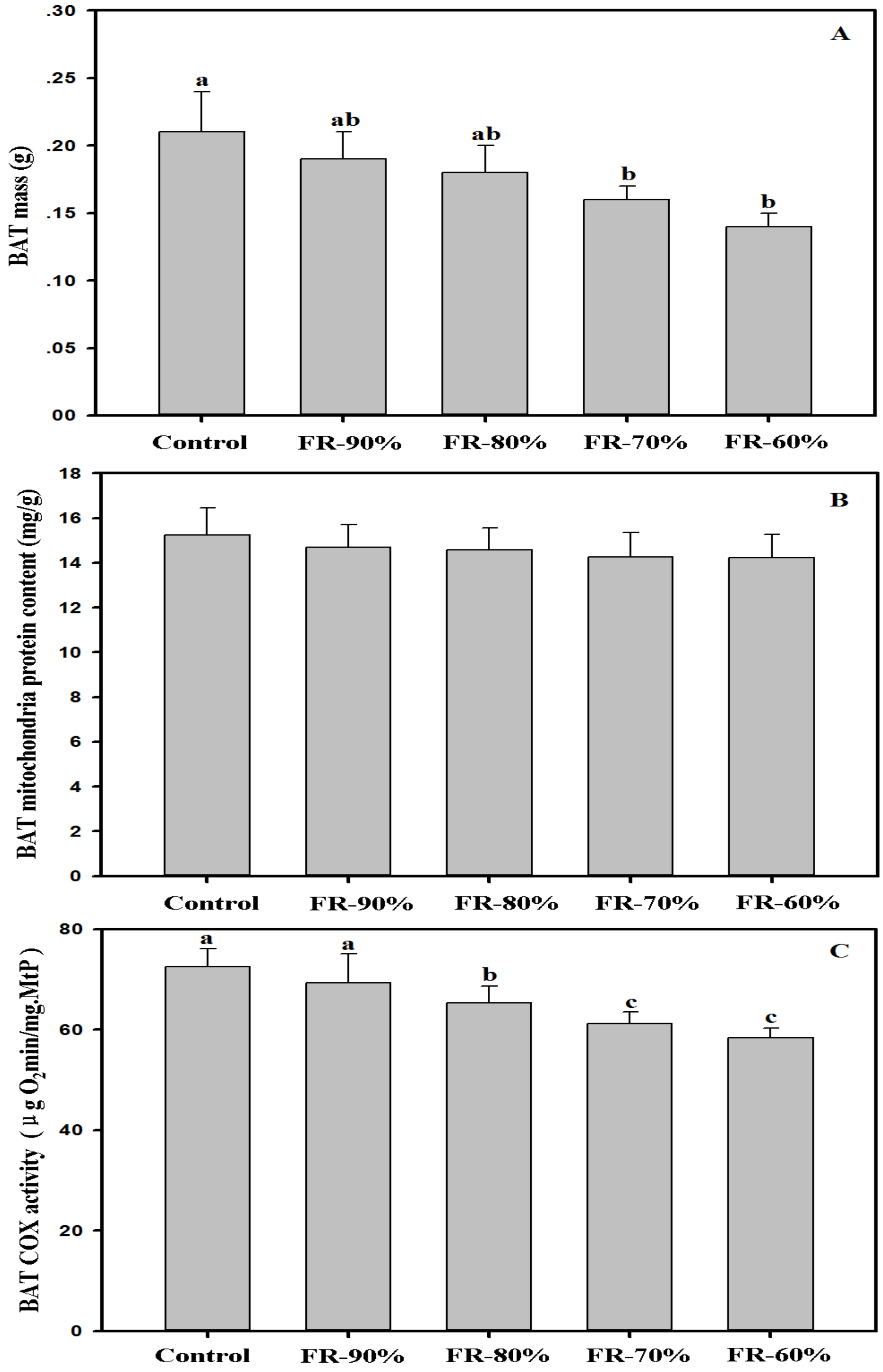

Figure 4: BAT mass (A), protein content of mitochondria (B) and COX activity (C) in *Apodemus chevrieri* during the course of food restriction. Different superscripts in each row indicate significant difference ($P<0.05$).