Changes of weight and microstructure of brown adipose tissue in *Tupaia belangeri* under cold acclimation

Zhang Lin¹,#, Zuo Mu-lin¹,#, Cheng Jin-long² and Zhu Wan-long¹*

¹Key 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
²Kunming Haikou forest farm, Kunming, 650000, China
*
Corresponding E-mail: zwl_8307@163.com
#Zhang L and Zuo ML are equal to this work

(Received: 20/01/16 ) (Accepted:18/08/16)

ABSTRACT

*Tupaia belangeri*, which were the unique tropic and subtropic small mammal and distributed in the south of China. In order to investigate the adaptation of brown adipose tissue (BAT) in *T. belangeri* under cold acclimation, weight and microstructure of BAT were measured. The results showed that after cold acclimation, the color of BAT in tree shrew deepened, the weight of BAT increased 5.83% and interscapular weight of BAT was 23.6% in total body fat mass; and the microstructure of BAT found that mitochondria diameter of BAT cell increased 43.4%, ridge count increased by 49.3% and was palisade arrangement, fat droplets become smaller, showing a pupil bubbly. These results indicate that the change of weight and microstructure of BAT were related to demand of the heat production in tree shrews under cold acclimation.

Keywords: *Tupaia belangeri*; Brown adipose tissue; Cold acclimation

INTRODUCTION

In mammals, adipose tissue exists in two forms, namely brown adipose tissue (BAT) and white adipose tissue (WAT). BAT on histology and function are different from WAT, BAT cells and mitochondria with more fat drops, rich blood supply and sympathetic innervation[1]. BAT is of great significance in the regulation of heat energy in the cold condition of newborn mammals and mammals, as well as the awakening from hibernation. BAT has been identified as the main site of non shivering heat production (NST), and UCP1 can only be separated from its cell membrane[2].

Research on the BAT, foreign scholars from anatomy, histology, physiology, cytology and biochemistry were extensive and detailed research, and the various affect factors such as age, body size, environmental temperature, food, photoperiod, reproduction and hormone etc. were studied[3]. Most of the studies in the laboratory domestication, under natural state in the field of Palaearctic animals and experimental animals, and the Oriental animal research is less, adapt to the changes in the ultrastructure of the cold has not been reported. To the Oriental typical small mammals of the tree shrew as experimental materials, weight and microstructure of BAT were studied under the cold acclimation for 28 days, for understanding of BAT how to adjust the structure of its response to cold stress, and adapt to the environment in which they live.

MATERIALS AND METHODS

Samples
Tree shrew (*T. belangeri*) were captured (25°25’~26°22’ N, 102°13’~102°57’E, 1679 m in altitude) around boscages
at Luquan County, Yunnan Province. After being captured, tree shrews were transported to the School of Life Science of Yunnan Normal University, Kunming, China (1910m in altitude). Animals were kept individually in wire cages (35×25×20 cm) with no bedding. The animals maintained under 12L:12D (light: dark, lights on 08:00) photoperiod and room temperature that were control under 25±1°C for 4 weeks. The cold-exposed animals were maintained under 5±1°C and 12L:12D (light: dark, lights on 08:00) photoperiod for 4 weeks. All pregnant, lactating or young individuals were excluded. After 28 day, all animals were sacrificed between 0900h and 1100h by decapitation, and the weight of BAT was measured.

**Measurement of microstructure of BAT**

The experimental tissue sections are divided into tissue sections and micro sections. Take cold acclimation 0d and 28d bat samples and then fixed in 10% neutral formalin, tissue section, H. E. staining. The electron microscope was made by the center of electron microscope of Kunming Medical University.

**Statistical analysis**

Using SPSS16.0 statistical software package to the relevant statistical processing. Under cold acclimation conditions, the differences between groups were analyzed by independent samples T test.

**RESULTS**

**Tissue dissection**

The anatomy of visible, tree shrew BAT mainly distributed in the shoulder, shoulder, neck and axillary parts. BAT is basically the same in the distribution of tree shrews and other small mammals in vivo. We use BAT (the relative weight percentage of BAT body weight) as a function of BAT standard index. After cold acclimation, tree shrew brown adipose tissue color deepened significantly (Figure 1) and interscapular at brown adipose tissue for body fat weight of 23.6% (Figure 2), brown adipose tissue relative weight increased 5.83%.

**Results of section microstructure**

Study on microstructure of tree shrew in brown adipose tissue and found that, bat has abundant capillaries and mitochondria and mature mitochondrial tends to rule of circular (Figure 3). After 28 days of cold acclimation, mitochondria became larger, brown adipose tissue mitochondria diameter increased 43.4%, and showed palisading, fat droplets become smaller, showing a pupil bubbly. Changes in the number of bat mitochondrial cristae mitochondrial size ratio can reflect the production of thermal activity, crest and the number of mitochondrial oxidative phosphorylation activity, oxidative phosphorylation activity high crest number more, after 28 days of cold acclimation, ridge count increased by 49.3%(Table 1).

**DISCUSSION**

Fats were found in all the body of tree shrew, which enrich in the surrounding subcutaneous and organs. White adipose tissue is widely distributed around the body subcutaneous tissue and organs; and brown adipose tissue mainly distributed in between the shoulder blades, back of the neck, armpits, mediastinum, and kidney around; and brown adipose tissue, white adipose tissue in some parts of the mixed, not easy to separate. The 28 cold acclimation, we found that the tree shrews of brown adipose tissue and white adipose tissue is more mixed, is difficult to separate. This is consistent with the adult female 129Sv mice [1].

The white part of the organ is mainly composed of white adipose tissue, and the brown part is composed of brown
adipose tissue. The combination of white fat cells and brown fat cells is controlled by genes, and depends on the following factors, including age, sex, environmental temperature, and nutritional status. This in Sv129 female mice were reported similar [4]: in certain parts of the subcutaneous posterior, mesentery and peritoneum) white fat cells were more in number, and in the other parts (anterior subcutaneous, mediastinal, abdomen and pelvis) brown fat cells are more. The white fat and brown fat are mixed together in the skeletal muscle that is closely linked to the limbs. A comparative study of Sv129 and B6 mice showed that Sv129 contained more brown adipose tissue.

Table 1 Effect of adipose cell mit. Under cold acclimation

<table>
<thead>
<tr>
<th></th>
<th>BAT</th>
<th>WAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mit. Diameter (µ)</td>
<td>0.46±0.01 0.66±0.03</td>
<td>0.43±0.04 0.51±0.02</td>
</tr>
<tr>
<td>Cristae numbers</td>
<td>5.31±0.18 7.93±0.28</td>
<td>4.81±0.27 5.3±0.38</td>
</tr>
</tbody>
</table>

Similar to the mouse Sv129. Compared with control group, the cold acclimation of Tupaia belangeri fat color deep, suggesting that more fat to brown adipose conversion [1]. This phenomenon is reversible, and in brown adipose tissue, the increase in the number of capillaries and nerve [5-7]. Theoretically, the newly formed brown fat may be derived from the existing stem cells, or stem cell migration, or directly from the white fat cells, or even the combination of several other forms. Reversible phenomenon of fat, or fat plasticity, has the following reasons [1]: 1.after cold acclimation, the white fat cells in the body tissues of the animals were reduced, and the content of the white fat cells decreased with the number of the newly born brown fat cells; 2.in the white adipose tissue, the vast majority (80-95%) of the newly formed brown adipose cells were negatively correlated with BrdU in the white adipose tissue after treatment with the 3-adrenergic receptor agonist; 3.after cold acclimation, although fat cell precursors in brown adipose tissue in a significant increase in the number, but no increase in white adipose tissue; 4.cold acclimation or 3-adrenergic receptor agonists after treatment in white adipose tissue and the formation of the new UCP1 positive brown fat cells showing intermediate features of white and brown fat cells; 5. separation performance of brown fat cells in the middle of a white and brown fat cells; 6. the morphology and protein expression of brown fat cells was related to norepinephrine level of organization.

During cold acclimation, mitochondrial size and crest number of BAT of tree shrew have taken place a series of changes, BAT mitochondrial mature tends to rule the round. The mitochondria was significantly larger, increasing the diameter of the mitochondria and crest was palisade arrangement, straddle the bubbly. Mitochondria have a cell “power plant”, said the final oxidation of sugar, fat and protein 3 nutrients in place is the mitochondrial inner membrane fold ridge. After the cold acclimation for 28 days, the number of mitochondria fat cells were increased, and the heat production needs. Senault found in cold acclimated rats, bat cells change is small, and the number
increased [8]; Thomson found rat mature bat mitochondria tended to be round, average diameter is 0.7 m, and white adipose mitochondrial diameter of only half of the bat [9]. Chaffee et al. also found that when rats were cold acclimation, BAT mitochondria became larger, and the number of ridges increased and tended to be in the grid. They believed that the more compact the mitochondrial ridge, the greater the heat capacity of BAT mitochondria[10]. General studies have also found that in rats exposed to cold, BAT experienced an increase in cell number, duplication, and supply to the blood [11]. Aleksiuik et al. found in red squirrels, structure of BAT in summer was similar to WAT, but more vacuole of cells in winter[12]. Cameron et al. also found that BAT cells were mostly vacuole cell in cold exposure in rats[11].

In conclusion, these results indicate that the change of weight and microstructure of BAT were related to demand of the heat production in tree shrews under cold acclimation.

Acknowledgements
This research was financially supported by National Science Foundation of China (No. 31260097; 31560126), and Project of Basilic Research of Yunnan Province (No. 2013FA014).

REFERENCES