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Journal of Thermal Biology 30 (2005) 431–436

Journal of THERMAL BIOLOGY

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Suppression of thermogenic capacity during reproduction in primiparous brandt's voles (*Microtus brandtii*)

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Received 1 December 2004; accepted 12 May 2005

Abstract

(1) To investigate the changes of brown adipose tissue (BAT) thermogenic capacity in primiparous Brandt's voles during different phases of reproduction, BAT weight, mitochondrial protein concentration, cytochrome c oxidase (COX) activity, and uncoupling protein (UCP1) contents were measured.
(2) Both cytochrome c oxidase activity and UCP1 contents decreased significantly during lactation, suggesting that thermogenic capacity was suppressed.
(3) The decrease of thermogenic capacity during reproduction, especially during lactation, is compensation to the large demand of energy for reproduction. This is advantageous for energy conservation and lactation in Brandt's voles. UCP1 is the base of molecular thermogenesis of BAT in Brandt's voles.

Keywords: Brown adipose tissue (BAT); Thermogenesis; Cytochrome c oxidase (COX); Uncoupling protein 1 (UCP1); Brandt's voles; *Microtus brandtii*

1. Introduction

Reproduction, especially lactation is a physiological process characterized by much energy demand due to the expenditure of energy storage to the production of milk (Degen et al., 2002). However, mammals have evolved a number of different reproductive strategies to ensure that offspring were born during a time of a year that provided maximum survival potential (Steger et al., 1985). Caloric intake plays a major role in reproduction, especially for small mammals (Degen et al., 2002; Bergallo and Magnusson, 1999; Weiner, 1987). Beside the increase in energy intake, energy requirement for reproduction by small mammals could be satisfied through reliance on energy reserves by decreasing other energy expenditures (Degen et al., 2002; Liu et al., 2003). As a main pathway of energy expenditure, thermogenesis plays an important role in energy balance during reproduction.

Nonshivering thermogenesis (NST) is an important mechanism for thermoregulatory heat production,

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0306-4565/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtherbio.2005.05.005

particularly in small, cold-acclimated mammals (Jansky, 1973). The main mechanism for NST in brown adipose tissue (BAT) is that the mitochondrial proton conductance pathway leads to an uncoupling of substrate oxidation from the synthesis of ATP. Uncoupling protein 1 (UCP1) plays a key role in molecular thermogenesis (Nicholls and Locke, 1984; Ricquier and Bouillaud, 2000). It has been widely accepted that high expression of UCP1 contents or mRNA expression may serve as an indicator of NST capacity, and therefore increase the energy expenditure (Xiao et al., 2004).

It was demonstrated that thermogenic capacity was significantly suppressed during lactation, but not pregnancy, in laboratory mice and rats (Trayhurn et al., 1982; Villiamson et al., 1986). However, energy intake showed an increase during pregnancy (Wade et al., 1986; Trayhurn and Richard, 1985; Trayhurn, 1989), which implied that diet-induced stimulation of BAT thermogenesis is suppressed in the pregnant animals (Trayhurn, 1989). But this is not the case in golden hamster (*Mesocricetus auratus*) and Djungarian hamsters (*Phodopus sungorus*). In these two species, the suppression of BAT thermogenesis occurred during pregnancy, to sustain lactation (Wade et al., 1986; Schneider and Wade, 1987). Thus, it seems that there are different species-specific thermogenic mechanisms among species or between field and laboratory animals. We hypothesized that there might exist a trade-off between thermogenesis and energy intake during reproduction. Many studies have been undertaken on thermogenesis changes during reproduction, including NST, Guanosine diphosphate protein binding (GDP-binding), cytochrome c oxidase (COX) activity and UCP mRNA expression (Trayhurn, 1983; Trayhurn et al., 1982; Schneider and Wade, 1987; Xiao et al., 2004). However, the quantification of UCP1 during different phases of reproduction was not clear. The aim of this research is to elucidate the molecular basis of thermogenesis during reproduction in Brandt's voles.

Brandt's voles (*Microtus brandtii*) are a typical steppe herbivore. They are distributed mainly in the Inner Mongolian grassland of China, the Republic of Mongolia, as well as in the region of Beigaer Lake in Russia (Zhang and Wang, 1998). Within their range, the climate is warm in summer and cold in winter. The average annual temperature is 0–4 °C and winter can last for more than 6 months. It had been found that Brandt's voles showed seasonal changes in basal metabolic rate (BMR) and nonshivering thermogenesis (NST) (Wang et al., 2003). The increase of NST in cold or winter could be reflected in the weight of BAT, mitochondrial protein contents and UCP1 mRNA levels (Rafael et al., 1985; Li et al., 2001). Reproduction on Brandt's voles lasts about 3 months from May to August (Zhang and Wang, 1998), and pregnant voles significantly increased body mass, energy intake, metabolizable energy intake, and higher lipid mass than controls, and followed by a decrease during lactation (Liu et al., 2003). Brandt's voles met most of their energy demand not only by increasing the energy intake, but also by mobilizing body reserves. We first report here the changes in cytochrome c oxidase (COX) activities and UCP1 contents during reproduction in primiparous Brandt's voles. Thermogenic responses at different phases of reproduction were also observed.

2. Materials and methods

2.1. Animals and experimental design

All subjects in this experiment were the offspring of a laboratory colony that started with Brandt's voles that were live-trapped in Inner Mongolia and transported to the Institute of Zoology, Chinese Academy of Sciences in Beijing. Subjects were maintained on standard rabbit chow, then separated in plastic cages at 23 ± 1 °C under long photoperiod (16L: 8D) with lights on at 04: 00 h. Food and water were available ad lib. Pups were separated from their mothers and fed in group after weaning. Control females were randomly chosen. Experimental females were paired randomly with males and checked vaginal embolus twice every day. Females were identified as pregnant once a vaginal embolus was noticed and were separated from males immediately. They were then raised individually in plastic cages ($30\,cm \times 15\,cm \times 20\,cm$). The experiment was carried out from May 2003 to August 2003.

2.2. Isolation of mitochondria and cytochrome c oxidase activities (COX) activity measurements

The interscapular BAT was removed from animals immediately after they were sacrificed, each pad was then weighted and trimmed connective tissue. Mitochondria were prepared as described by Wiesinger et al. (1989) and the protein concentrations were determined by the method of Folin phenol method (Lowry et al., 1951) with bovine serum album as standard.

COX of BAT was measured at 25 °C in 1.96 ml of respiration medium (100 mM KCl, 20 mM TES, 1 mM EGTA, 2 mM $MgCl_2$, 4 mM KH_2PO_4, 60 μM BSA, pH7.2) with a Clark electrode (Hansatech Instruments LTD, England, DW-1). 10 μl aliquot taken from the supernatant and 30 μl cytochrome c (37.9 mg/ml) were added to the electrode and the COX were measured in a final volume of 2 ml.The activity of cytochrome c oxidase (COX) was expressed as nmol O_2/min per mg mitochondrial protein (Wiesinger et al., 1989).

2.3. Western blot of UCP1

Total BAT protein (20 μg per lane) was separated in a discontinuous SDS-polyacylamide 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 transfer with Coommassie brilliant blue and Ponceau red, respectively. UCP1 was detected using a polyclonal rabbit antibody against Djunrarian hamster UCP1 (1:5000) as primary antibodies (supplied by Dr. M. Klingenspor, Department of Biology, Phillips University Marburg, Germany) and sheep anti-rabbit (Sigma, 1:5000) as secondly antibodies (Klingenspor et al., 1996). We used enhanced chemoluminescence kit (ECL, Amersham) as detection system and unspecific binding sites were saturated with 5% degreased milk in PBS. UCP1 concentration was expressed as relative units (RU), and quantified by using the Scion Image.

2.4. Statistics

Data were analyzed using SPSS package (SPSS 1998). Distributions of all variables were tested for normality using the Kolmogorov–Smirnov test. Abnormally distributed data were transformed to natural logarithms to normalization. One-way ANOVA followed by a post-hoc LSD test was used to compare different responses between different phases of reproduction. All values are expressed as mean ± SE, and $p<0.05$ was taken to be statistically significant.

3. Results

3.1. Body mass

There was a significant change in body mass during different phases of reproduction (One-way ANOVA, $F(3,23)=49.470$, $P<0.01$, Table 1). Brandt's voles gained body mass during pregnancy, then lost body mass significantly after parturition, and reached the original mass during weaning. Body mass of non-reproductive group was 49.7 ± 2.7 g. During pregnancy, body mass of voles increased by 60.0%, which was significantly higher than that of any other groups (LSD, $P<0.01$). During lactation, the body mass was significantly decreased compared with pregnancy and at weaning, body mass declined to a level which was no different with that of non-reproductive group (LSD, $P>0.05$).

3.2. Brown adipose tissue (BAT)

Absolute mass of interscapular BAT showed no significant changes during reproduction (One-way ANOVA, $F(3,23)=0.763$, $P=0.527$, Table 1). Relative mass of BAT (mg/g body mass) also showed no significant changes during reproduction (One-way ANOVA, $F(3,23)=0.582$, $P=0.633$, Table 1).

3.3. Mitochondrial protein content

Absolute mitochondrial protein content showed no significant changes during different phases of reproduction (One-way ANOVA, $F(3,23)=1.658$, $P=0.204$, Table 1). However, relative mitochondrial protein content showed significant changes during reproduction (One-way ANOVA, $F(3,23)=4.672$, $P<0.05$); it was higher during the period of lactation than in any other groups (LSD, $P<0.05$).

3.4. Cytochrome c oxidase (COX) activity

COX activity showed significant variations in different reproductive phases (One-way ANOVA, $F(3,23)=5.047$, $P<0.01$, Table 1). COX of lactation (88.18 ± 12.47 nmolO_2/min.mg mitochondrial protein)

Table 1
Body mass and thermogenic parameters during different phases of reproduction in primiparous Brandt's voles (*Microtus brandtii*)

	Control	Pregnancy	Lactation	Weaning	*P* value
Sample size	8	6	6	7	
Body mass (g)	49.6 ± 2.7[a]	79.5 ± 2.1[b]	50.5 ± 2.3[a]	47.4 ± 1.6[a]	0.01
BAT mass					
g of total tissue	0.16 ± 0.02	0.18 ± 0.03	0.13 ± 0.03	0.14 ± 0.02	ns
mg/g body mass (%)	3.18 ± 0.51	2.31 ± 0.37	2.61 ± 0.47	3.01 ± 0.34	ns
Mitochondrial protein in BAT					
mg of total tissue	2.08 ± 0.22	1.76 ± 0.18	2.51 ± 0.44	1.85 ± 0.11	ns
mg/g BAT	14.49 ± 0.88[a]	10.55 ± 1.24[a]	20.86 ± 2.51[b]	13.82 ± 1.59[a]	0.05
Cytochrome c oxidase activity					
(nmolO_2/min mg mitochondrial protein)	128.43 ± 9.92[a]	144.00 ± 6.00[a]	88.18 ± 12.47[b]	129.99 ± 11.74[a]	0.01

Values are expressed as mean ± SE.
Different superscripts in the same row indicate difference significantly ($P<0.05$).

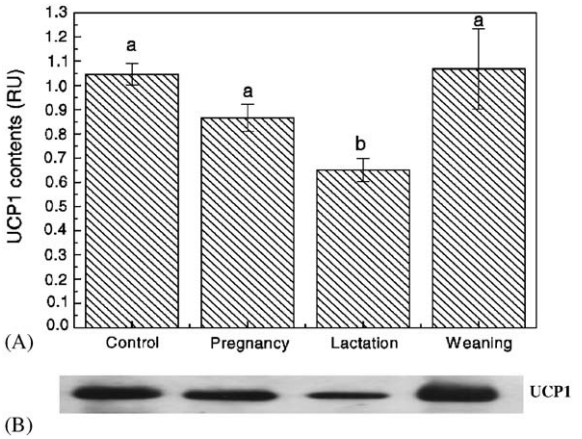

Fig. 1. (A) Changes of uncoupling protein 1 (UCP1) contents in interscapular brown adipose tissue during different phases of reproduction in primiparous Brandt's voles (*Microtus brandtii*). UCP1 content during lactation was lower than any other groups. Other three groups showed no significant differences. One-way ANOVA followed by a post-hoc LSD test was used to detect the differences among different phases of reproduction. All values are expressed as mean ± SE, and $p<0.05$ was taken to be statistically significant. Columns with different letters indicate difference significantly. (B) Representative western bloting band of UCP1 in brown adipose tissue.

showed 31.3%, 38.8% and 32.2% lower than that of non-reproduction (128.43 ± 9.92 nmolO_2/min.mg mitochondrial protein), pregnancy (144.00 ± 6.00 nmolO_2/min.mg mitochondrial protein) and weaning group (129.99 ± 11.74 nmolO_2/min.mg mitochondrial protein), respectively (LSD, $P<0.05$, Table 1).

3.5. Uncoupling protein 1 (UCP1)

UCP1 content during lactation was the lowest (0.64 ± 0.05) (RU) (One-way ANOVA, $F(3,23)=3.109$, $P<0.05$, Fig. 1). Other three groups showed no significant differences in UCP1 contents (LSD, $P>0.05$). UCP1 content decreased by 36.4%, 18.9%, and 38.4% compared with non-reproductive (1.00 ± 0.06(RU)), pregnancy (0.78 ± 0.05(RU)), and weaning (1.03 ± 0.18(RU)) groups, respectively (Fig. 1).

4. Discussion

Our results indicated that body mass of Brandt's voles increased during pregnancy and decreased during lactation, consistent with the results of Liu et al. (2003). Degen et al. (2002) also found that changes of body mass during reproduction in common spiny mice (*Acomys cahirinus*) displayed the similar trend. During pregnancy and lactation, small mammals' nutritional requirements increased and maintainance of appropriate body mass is critical to the survival of a species (Amico et al., 1998). To maintain a stable body mass, energy intake and expenditure must be in balance. Detailed energy balance studies have been conducted for several species, such as mice (Richard and Trayhurn, 1985), golden hamsters (Wade et al., 1986), and Djungarian hamsters (Schneider and Wade, 1987; Trayhurn, 1988). In many small mammals, significant increases in body mass during pregnancy was mainly caused by the deposition of body fat (Trayhurn, 1988; Degen et al., 2002; Liu et al., 2003). Lactation is an energetically expensive process for small mammals. It imposes nutritional stress to maternal metabolism (Williamson, 1980), and the utilization of body fat reserves could cause a decrease in body mass during lactation for small mammals. An appropriate body mass is essential to puberty and reproduction for animals (Frisch and McArther, 1974; Michael and Castracane, 2000; Spicer, 2001), which is monitored by leptin, a signal that appears to be widespread among vertebrates and plays a variety of roles, including decreasing of food intake and increasing of energy expenditure (Michael and Castracane, 2000; Spicer, 2001).

BAT is the main site for NST in small mammals. The adaptive changes in BAT were achieved through modifications in the mass of BAT, mitochondrial contents and the concentrations of UCP1 (Trayhurn et al., 1982; Martin et al., 1989; Xiao et al., 2004). Our results showed that COX activity and UCP1 both decreased significantly during lactation, and decreased only slightly during pregnancy, suggesting that thermogenic capacity was suppressed during reproduction in Brandt's voles. Similar results were also found in other animals. Trayhurn (1983) found that NST decreased in early and mid-lactation in mice, which also occurred at the time of weaning, but one week later after weaning, NST returned to the level during non-reproduction. During pregnancy and lactation, mice reduced COX and GDP binding (Trayhurn, 1982; Villiamson et al., 1986). Martin et al (1989) found UCP1 mRNA expression of mice in pregnancy decreased by 15% compared with virginal controls, and remained at the low levels throughout pregnancy and lactation, but then increased significantly after weaning. Xiao et al. (2004) documented that UCP1 decreased by 70% in lactation compared with controls. Similar to UCP1, UCP3 mRNA and protein content in BAT also decreased during lactation in mice and rats (Pedraza et al., 2001; Xiao et al., 2004).

Despite some studies in mice and rats, we still know little about thermogenic capacity during reproduction in small rodents, especially UCP1 changes of BAT during reproduction in field small mammal. Wade et al. (1986) and Schneider and Wade (1987) found that both Syrian hamsters (*Mesocricetus auratus*) and Djungarian

hamsters suppressed BAT thermogenesis in pregnancy and lactation. We also found a significant reduction of COX and UCP1 during lactation in Brandt's voles, but it showed no significant decrease during pregnancy. The energy stores in pregnant Brandt's voles were mainly dependent on the energy intake (Liu et al., 2003) and, however, the increasing food intake was not enough for energy expenditure during lactation, and the suppression of thermogenesis was a distinct physiological adaptation for energy conservation (Trayhurn, 1982; Trayhurn and Richard, 1985; Trayhurn, 1989; Xiao et al., 2004). UCP1 played a key role in the basis of molecular thermogenesis of BAT in Brandt's voles. The suppression of BAT thermogenesis was a physiological adaptation for energy conservation, and it is advantageous to meet challenges of natural environment (Trayhurn, 1982; Schneider and Wade, 1987; Ricquier and Bouillaud, 2000).

5. Summary

COX and UCP1 contents in Brandt's voles were suppressed during reproduction, especially during lactation. This is a physiological adaptation for energy conservation to compensate the negative energy balance. UCP1 played a key role in the basis of molecular thermogenesis of BAT in Brandt's voles.

Acknowledgements

We wish to thank Dr. Martin Klingenspor, Department of Biology, Philipps-University Marburg, Germany, for providing the hamster UCP1 antibody. Thank Dr. Ming Yang, Institute of Zoology, Chinese Academy of Sciences, for helping the UCP1 measurement and Dr. Zuoxin Wang, Department of Psychology, Florida State University, for reading the draft and giving some suggestions. This study was financially supported by the National Natural Science Foundation of China (Nos. 30430140 and 30170151), the State Key Laboratory of Integrated Management of Pest Insects and Rodents (IMP0302 and IMP0405), and the Chinese Academy Sciences (No. KSCX2-SW-103).

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