Physiology & Behavior 118 (2013) 189–194

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Competition in newborn rabbits for thermally advantageous positions in the litter huddle is associated with individual differences in brown fat metabolism

Amando Bautista [a,*], Francisco Castelán [a], Humberto Pérez-Roldán [b], Margarita Martínez-Gómez [a,c], Robyn Hudson [c,**]

[a] Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, km 1.5 Carretera Tlaxcala-Puebla S/N, AP 262 Tlaxcala, Mexico
[b] Maestría en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, km 1.5 Carretera Tlaxcala-Puebla S/N, AP 262 Tlaxcala, Mexico
[c] Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, AP 70228 DF, Mexico

HIGHLIGHTS

- Lighter rabbit pups occupy the most peripheral positions in the litter huddle.
- They have lower body temperatures, and poorer milk conversion, survival and growth.
- They show greater expression of uncoupling protein 1 (UCP-1).
- Via sympathetic activation, UCP-1 is essential for non-shivering thermogenesis.
- Lighter pups may experience greater sympathetic activation during early development.

ARTICLE INFO

Article history:
Received 20 September 2012
Received in revised form 2 February 2013
Accepted 17 May 2013

Keywords:
Thermoregulation
Brown adipose tissue
UCP-1
Development
Individuality
Oryctolagus cuniculus

ABSTRACT

The altricial young of the European rabbit (*Oryctolagus cuniculus*) are not brooded by the mother, and although they are born into an underground nest, depend importantly on the warmth and insulation provided by littermates for their early growth and survival. Consistent with previous studies, heavier pups occupied more central, thermally advantageous positions in the litter huddle, maintained higher body temperatures, obtained more milk, were more efficient at converting it to body mass, and consequently grew faster than their lighter sibs occupying the periphery of the huddle. In the present study we measured the expression of uncoupling protein 1 (UCP-1), which is essential for the metabolism of brown adipose tissue to generate body heat in response to cold. In nine litters of domestic rabbits maintained for the first four postnatal days at temperatures below their critical thermoneutral temperature, peripheral pups showed greater expression of UCP-1 than intermediate pups, and these greater expression than central pups. This suggests that during early development littermates of the rabbit experience differing degrees of activation of the sympathetic nervous system as a consequence of exposure to different thermal environments associated with different positions in the litter huddle. Whether this is associated with long term differences in the physiological response to cold and perhaps in the manner of responding to other environmental challenges is currently under investigation.

1. Introduction

One of the most important challenges facing newborn mammals is the need to maintain an adequate body temperature for survival and for (often rapid) growth. For altricial young born without fur, with a high body surface to body mass ratio, and without the ability for shivering thermogenesis, the challenge is particularly great [1–3]. Although many altricial mammals are born into the shelter of a den, nursery burrow or nest, during the mother's absence and periods of low ambient temperature they depend critically on behavioral thermoregulation such as huddling with littermates [4–8]. They also depend on non-shivering thermogenesis fueled by the burning of brown adipose tissue (BAT), of which many species have a prominent and richly vascularized and innervated supply ([9,10]; reviews in Ref. [6,11]).

The European rabbit (*Oryctolagus cuniculus*), including its domesticated (laboratory) form, is a good example. The altricial, naked

* Corresponding author. Tel./fax: +52 246 123775.
** Corresponding author. Tel./fax: +52 55 5622 3828.
E-mail addresses: abopup@gmail.com (A. Bautista), rhudson@biomedicas.unam.mx (R. Hudson).

0031-9384/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.physbeh.2013.05.035

young are born into a nest of grass and fur constructed by the mother in a nursery burrow (or laboratory nest box). Immediately after giving birth the mother leaves the young and only returns for a few minutes approximately once every 24 h to nurse ([12–16]; reviews in Refs. [17,18]). In the wild, however, ambient temperatures in the nest burrow can be considerably lower than the newborn pups' critical thermoneutral temperature of approximately 35 °C [2,19,20]. From birth the pups perceive fine differences in environmental temperatures, and when placed on a thermal gradient quickly locate and come to rest in their thermoneutral zone [14,21–23]. The newborn pups depend critically on the presence of littermates for warmth and insulation [4,7,8,20], and like the altricial young of other species ([5,24]; review in Ref. [6]), at low ambient temperatures form a tight, insulating huddle [4,7,8,14]. Positions in the huddle, however, are not equally distributed and higher body mass young consistently occupy central, thermally more advantageous positions than their lighter sibs ([25–28] for similar findings in rats). Moreover, heavier, central pups maintain higher body temperatures, obtain more milk, are more efficient at converting it to body mass, and consequently show faster growth and a higher probability of survival than their lighter, peripheral sibs (reviews in Refs. [29,30]).

Like many other altricial mammals, rabbit pups are born with a notable supply of BAT. It makes up an estimated 5% of their body mass and comprises about half their total body fat [9]. Whereas during the first few postnatal days pups have only rudimentary or no capacity for shivering thermogenesis, they can rapidly mobilize BAT for non-shivering thermogenesis via the local release of noradrenaline at sympathetic nerve endings in response to cold ([10,31]; review in Ref. [11]). Via a well documented chain of biochemical events, adrenergic signaling at the cell membrane of brown adipocytes results in the activation of uncoupling protein 1 (UCP-1), leading to a proton electrochemical gradient across the inner membrane of mitochondria to produce heat (review in Ref. [11]). Thus, UCP-1 expression is required for cold-induced thermogenesis in BAT.

It was therefore the aim of the present study to measure the expression of UCP-1 in the BAT of rabbit pups occupying central, intermediate and peripheral positions in the litter huddle during the first four postnatal days when nest mortality and vulnerability to hypothermia are greatest [4,7,32–35]. We expected that central pups would be heavier at birth, would have higher body temperatures, obtain more milk, and convert it more efficiently into body mass than their more peripheral sibs. We further expected that they would show lower expression of UCP-1, indicating lower mobilization of BAT for non-shivering thermogenesis.

2. Materials and methods

2.1. Animals

We used nine litters of chinchilla-strain domestic rabbits from nine different females each mated with a different male, and bred and maintained at the Centro Tlaxcala de Biología de la Conducta, Tlaxcala, Mexico. The females were kept in individual stainless steel cages (90 × 60 × 40 cm height) and on a 16:8 h light:dark cycle to approximate conditions at the height of the summer breeding season for wild rabbits in Europe. They were between 10 and 14 months old and had given birth to at least one previous litter. Ambient temperature was between 17 and 24 °C, and food (Conejina, Purina, Mexico) and water were continuously available. For nest building, several days before term females were given hay and an open-top wooden box (40 × 35 × 15 cm height) lined with wood shavings.

2.2. Procedure

Experimental animals were kept and treated according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA, and the National Guide for the Production, Care and Use of Laboratory Animals, Mexico (Norma Oficial Mexicana NOM-062-200-1999).

2.2.1. Day 0

On the day of birth the nest box was removed from the mother's cage, the pups were weighed on an electronic balance (Ohaus, Navigator) to the nearest 0.1 g, litters were culled to the eight heaviest pups, and in random order each pup (dark gray in this breed) was numbered on the back, flanks and ventrum with white correcting fluid (Nukote, Pelikan [25,27]). Litters were culled to eight as this number can usually survive the considerable early mortality in this species (cf. [32–35]) but still results in competition for thermally advantageous positions at the center of the litter huddle and in stable differences among littermates in obtaining these [27]. The pups were then returned in their nest box to the mother's cage to allow one undisturbed nursing.

2.2.2. Day 1

The following day at 09:00 h the nest box was taken from the mother's cage, the nest material removed to allow observation of the pups' behavior, and the box lined with flannel. To keep the pups in the center of the box and prevent them becoming trapped in the corners we placed them inside a 28-cm-diameter wire mesh hoop [25,27,28]. The box with the pups was placed in a cold room in continuous light with the temperature set at 25 °C (Oregon Scientific electronic thermometer, EMR963HG). This temperature, below the approximately 35 °C critical thermoneutral temperature for newborn rabbits [2,19,21,23,36], induces them to huddle but without compromising pup survival [7]. In nature, ambient temperatures in the nest chamber can drop below pups' thermoneutral range and well below the temperatures maintained here [20]. To eliminate possible drafts, the nest box was placed beneath a transparent acrylic cover (45 × 45 × 70 cm height), with a closed circuit video camera (SNC Networks) mounted in the roof of the box and connected to a computer outside the cold room to record pups' behavior [27]. The litter was filmed for 15 min ten times during 24 h (at 09:45, 11:45, 13:45. 15:45, 17:45; 21:45, 23:45, 01:45, 03:45, and 05:45 h), and body temperature measured four times (at 10:00, 14:30. 16:30, and 18:00 h). For this, in random order, the skin temperature of each pup was measured at the nape and groin representing high and low temperatures on the body surface, respectively [7,25], using a quick-reading Schultheis-type mercury thermometer (Millar and Weber T-6000). The mean of these two measures was used for all further calculations. Although skin temperature cannot be considered an accurate measure of core temperature (review in Ref. [6]) it gives consistent recordings of relative differences in body temperature among pups [4,7,26,27], which was the main interest in the present study. Measuring temperature took about 2 min per pup, after which the pup was weighed and returned to the nest box to minimize cooling of littermates.

2.2.3. Days 2 to 4

We followed the same procedure as on day 1 except that at 11:30 h the pups were induced to urinate in random order (ensured using a lottery of numbered cards) by lightly brushing their genital area with a finger, were weighed and returned to the observation box. Urination was induced to enable accurate measurement of milk intake after nursing 30 min later. At 12:00 h we removed the mesh hoop without disturbing the huddle and placed the mother beside the box so that she could jump in for the approximately 3 min nursing [25,27,37,38]. Immediately the mother jumped out of the box signaling the end of nursing we weighed the pups individually and placed them back in the box inside the hoop. We took the difference between their pre- and postnursing weight as a measure of milk intake.