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Differential metabolism of brown adipose tissue in newborn rabbits in relation to position in the litter huddle

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ABSTRACT

Competition for resources can contribute importantly to the early development of individual differences in behavioral and physiological phenotypes. In newborn rabbits, littermates compete for thermally favorable positions within the litter huddle. As brown adipose tissue (BAT) is the principal site of thermogenesis in such altricial young, we investigated differences in rabbit pups' growth and morphological differences in BAT associated with position within the huddle. We formed three treatment groups (7 litters/group): GI—birth (pups killed at birth); GII—chronic thermal challenge (pups killed after exposure to a moderately cold environmental during postnatal days 1–3); GIII—acute thermal challenge (as for GII but pups killed after an additional 30 min exposure to a very cold environment on postnatal day 3). Interscapular BAT was removed at death for histological analysis, and triglyceride concentrations measured in serum. Pups occupying central positions in the huddle had higher skin temperatures, obtained more milk, and were more efficient at converting this into body mass, than pups occupying peripheral positions. There was no significant difference in BAT morphology or triglyceride concentrations between pups at birth, nor between central and peripheral pups chronically exposed to moderate cold until postnatal day 3. However, during acute cold exposure at this age, peripheral pups were less able to maintain their body temperature, they depleted BAT fat reserves almost completely, and they had lower serum concentrations of triglycerides than central pups. These findings confirm the contribution of early sibling relations to individual differences in growth and metabolic processes associated with thermoregulation in newborn rabbits.

1. Introduction

Apart from humans (e.g. Sulloway, 2010), surprisingly little attention has been given to the role of siblings (or half siblings) in shaping individual differences in mammalian development, and even though in many species the young spend more time with their siblings than with their parents (reviews in Mock and Parker, 1997; Drummond, 2006; Hudson and Trillmich, 2008; Hudson et al., 2011a). Moreover, notable differences among littermates in physiology, behavior and probability of survival are often present from birth (Hudson and Trillmich, 2008; Hudson et al., 2011a; Bautista et al., 2015). In newborn rabbits, for example, heavier young or those from smaller litters are more likely to survive the first critical postnatal period, they typically obtain more milk, are more efficient at converting this into body mass, show more rapid motor development, and have higher levels of testosterone (Drummond et al., 2000; Muciño et al., 2009; Hudson et al., 2011a, 2011b; for wild rabbits see Rödel et al., 2008a,b, 2009a). They also typically occupy more central, thermally advantageous positions in the litter huddle, while spending less time in presumably costly efforts to gain these by climbing over or pushing between littermates (Bautista et al., 2008, 2013; Rödel et al., 2008a; Hudson et al., 2011a). Consistent with central pups having to invest less energy to maintain an adequate body temperature, they also have lower expression of uncoupling protein-1 (UCP-1), necessary for metabolizing triglycerides in brown adipose tissue (BAT) (Bautista et al., 2013), which under noradrenergic control newborn mammals activate to produce heat by non-shivering thermogenesis (Blumberg and Sokoloff, 1998; Cannon and Nedergaard, 2004).

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BAT thermogenesis is an important component of adaptive thermogenesis (Himms-Hagen, 1985; Cannon and Nedergaard, 2004). BAT, the major thermogenic center in rabbit pups (Hull and Segal, 1965a, 1965b; Gilbert et al., 2012), is composed primarily of brown adipocytes that are characterized by multilocular lipid droplets, a large number of mitochondria, dense sympathetic nervous innervation, and abundant UCP-1 (Loncar, 1991; Xiao et al., 2007). During cold exposure UCP-1 is up-regulated in BAT, which in turn generates heat produced by fatty acid oxidation (Cannon and Nedergaard, 2004, 2011; Liang and Ward, 2006). This heat is distributed throughout the body via the circulatory system, providing an effective mechanism for the maintenance of core body temperature when an animal is exposed to cold (Blumberg and Sokoloff, 1998; Vollmer and Skott, 2002; Cannon and Nedergaard, 2004; Seale et al., 2007).

In addition, it has been shown that by activating BAT via short-term cold exposure, triglycerides (TGL) are efficiently channeled into BAT (Bartelt et al., 2011, 2012). TGL are the main oxidizable substrate for thermogenesis and represent a major uptake process by BAT. Thus, serum TGL clearance is directly related to BAT activity (Bartelt et al., 2011). In response to acute cold exposure specific uptake of TGL by BAT is increased, heat is produced and transferred to the blood, leading to the maintenance of body temperature (Bartelt et al., 2011, 2012).

In the present study we examined if the differences in growth and survival of central and peripheral rabbit pups in the litter huddle described above are also associated with individual differences in morphological and physiological indicators of BAT thermogenesis during chronic and acute cold exposure. We examined thermogenesis during the first three postnatal days, as this is the period of highest postnatal mortality in rabbit pups (Drummond et al., 2000; Coureaud et al., 2000a,b). In addition, as the pups are almost furless at this age and have a high body surface to body mass ratio, they are particularly susceptible to hypothermia (Bautista et al., 2003; Gilbert et al., 2007, 2012).

2. Material and methods

2.1. Animals

We collected data from chinchilla-strain domestic rabbits (*Oryctolagus cuniculus*) bred and maintained at the Centro Tlaxcala de Biología de la Conducta, Tlaxcala, Mexico. We used 21 litters (culled if larger than 7 pups to reduce the probability of mortality during the study period; see Sections 2.2.2 and 2.2.3) from 19 different females, each mated with one of 13 different males. The females were between 6 and 18 months old, 4 were multiparous and 15 primiparous. They were kept in individual stainless steel cages ($90 \times 60 \times 40$ cm height), under fluorescent lights and on a 16:8 h light/dark cycle to approximate conditions at the height of the summer breeding season for rabbits in Europe. Ambient temperature was maintained between 18 and 20 °C, and water and food (Purina rabbit chow) were always available. For nest building, hay and an open-top wooden box $40 \times 35 \times 15$ cm height and lined with wood shavings were placed in the females' cages two days before term.

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. Group I: Birth

We used 7 litters removed from the mother at birth to minimize postnatal contact with cold before weighing and tissue sampling (total of 63 pups, mean litter size 9.0, SD 3.78). In our experience removal of the young has no ill effects on the females, presumably because early loss of whole litters is not uncommon in nature (Rödel et al., 2009b). In addition, these females were often used as foster mothers in concurrent experiments. Pups' body mass was recorded immediately to the nearest 0.1 g using an electronic balance, and the heaviest and the lightest pup were selected from each litter as an approximation for the positions they would have most likely occupied in the litter huddle as central or peripheral pups, respectively (see Sections 1 and 2.3.1). The pups were killed by decapitation, the trunk blood collected in polypropylene tubes, centrifuged (10,000 rpm/15 min), and the serum stored at −30 °C in a freezer (REVCO) until measurement of TGL concentrations (see Section 2.3.5.). The left interscapular pad of BAT was removed and immediately immersion-fixed for 24 h in Bouin–Duboscq solution in preparation for morphometric analysis (see Section 2.3.4).

2.2.2. Group II: Chronic exposure to moderate cold

We used 7 litters (total of 45 pups, mean litter size 6.42, SD 0.78, range 5–10, culled to a maximum of 7 pups; see Section 2.1) as we wanted to have litter sizes within the usual range for this chinchilla breed. Following procedures used in previous studies in our laboratory (Bautista et al., 2008, 2013; Reyes-Meza et al., 2011) on the day of birth the nest box was removed from the mother's cage, the pups were weighed on an electronic balance to the nearest 0.1 g, and in random order ensured using a lottery of numbered cards, each pup (dark gray in this breed) was numbered on the back, flanks and ventrum with white correcting fluid (Nukote, Pelikan). The pups were then returned in their nest box to the mother's cage to allow one undisturbed nursing.

Day 1: The following day at 08:30 h the nest box was taken from the mother's cage, the pups were weighed, their body temperature measured (see below), the nest material was removed to allow observation of the pups' behavior, and the box was 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 (Bautista et al., 2008, 2013; Reyes-Meza et al., 2011). The box with the pups was placed in a cold room in continuous light with the temperature set at 20 °C (Oregon Scientific electronic thermometer EMR963HG). This temperature, below the approximately 35 °C critical thermoneutral temperature for newborn rabbits (Dawkins and Hull, 1964; Hull, 1965; Várnai et al., 1970; Satinoff et al., 1976; Sokal and Sinclair, 1976; Pacheco-Cobos et al., 2003), induces them to huddle but without compromising pup survival when kept together with littermates (Bautista et al., 2003). In nature, ambient temperatures in the nest chamber can drop below pups' thermoneutral range and well below the temperature maintained here (Rödel et al., 2008c). To eliminate possible drafts, the nest box was placed beneath a transparent acrylic cover ($45 \times 45 \times 70$ cm height), with a closed circuit video camera (Vivotec Inc., San José, CA, USA) mounted in the roof of the box and connected to a computer outside the cold room to record pups' behavior (Reyes-Meza et al., 2011; Bautista et al., 2013).

The litter was filmed for 10 min 10 times during 24 hours (at 09:00, 11:00, 13:00. 15:00, 17:00, 23:00, 01:00, 03:00, 05:00, 07:00 h), and body temperature measured 5 times (at 09:00, 11:00, 13:00, 15:00, 17:00 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, using a quick-reading Schultheis-type mercury thermometer (resolution 0.2 °C; Millar and Weber T-6000, NY, USA).