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Growth axis maturation is linked to nutrition, growth and developmental rate

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ABSTRACT

Maturation of the mammalian growth axis is thought to be linked to the transition from fetal to postnatal life at birth. However, in an altricial marsupial, the tammar wallaby (*Macropus eugenii*), this process occurs many months after birth but at a time when the young is at a similar developmental stage to that of neonatal eutherian mammals. Here we manipulate growth rates and demonstrate in *slow*, *normal* and *fast* growing tammar young that nutrition and growth rate affect the time of maturation of the growth axis. Maturation of GH/IGF-I axis components occurred earlier in fast growing young, which had significantly increased hepatic *GHR*, *IGF1* and *IGFALS* expression, plasma IGF-I concentrations, and significantly decreased plasma GH concentrations compared to age-matched normal young. These data support the hypothesis that the time of maturation of the growth axis depends on the growth rate and maturity of the young, which can be accelerated by changing their nutritional status.

1. Introduction

In mammals, fetal growth relies on the coordinated action of several hormones, including the insulin-like growth factors (IGF-I and IGF-II) and their binding proteins (IGFBPs). IGF-I and IGF-II are expressed in most tissues and stimulate growth by autocrine or paracrine action (Boisclair et al., 2001; Randhawa and Cohen, 2005; Varela-Nieto and Chowen, 2005). IGFBPs modulate IGF function by controlling the accessibility of IGFs to their receptors and/or delaying their degradation (Cohick and Clemmons, 1993; Rajaram et al., 1997).

A crucial event in the maturation of the endocrine growth hormone insulin-like growth factor system (GH/IGF-I axis) is the up-regulation of growth hormone receptors (GHRs) in the liver (Breier et al., 1994; Gluckman et al., 1983; Maes et al., 1983; Schnoebelen-Combes et al., 1996). Early in development, cellular IGF production is largely regulated by the relative availability of nutrients (D'ercole et al., 1980; Gluckman and Pinal, 2003). Binding of GH to hepatic GHRs stimulates synthesis of both IGF-I and IGF-acid labile subunit (IGFALS) (Bichell et al., 1992; Ooi et al., 1997). IGFALS forms a ternary complex with IGF-I and IGFBP3 which extends the half-life and bioavailability of IGF-I (Boisclair et al., 2001). IGF-I also provides an inhibitory feedback signal via the hypothalamus and pituitary to affect GH synthesis (Berelowitz et al., 1981; LeRoith et al., 2001), so the brain plays a part in the regulation of further somatic growth.

The maturation of the endocrine growth axis begins around the perinatal period or after birth, depending on the mammalian species, and extends until the first weeks or months of postnatal life. In the few species examined, it is correlated with an increase in hepatic GHR production (Breier et al., 1994; Gluckman and Pinal, 2003; Schnoebelen-Combes et al., 1996). In sheep, a precocial mammal, fetal hepatic *GHR* and *IGF1* mRNA expression increase after birth in parallel with a rise of perinatal cortisol levels (Li et al., 1996). Hepatic *ALS* expression also increases abruptly from day 130 of fetal life until the first week of postnatal life (Rhoads et al., 2000a). Increased hepatic expression of *IGF1* and *IGFALS* result from increased hepatic GH/GHR signaling (Li et al., 1999). In contrast, in mice and rats, that are not precocial, similar changes in the expression of growth axis components do not occur until about 10 days after birth (Maes et al., 1983). At this stage the pups are developmentally equivalent to newborn precocial species such as sheep or horses. Their eyes are open, their bodies are covered in fur, they have regular coordinated movement and can eat a variety of food (Lohmiller and Swing, 2006). Hepatic *GHR* mRNA expression, GH binding, and plasma IGF-I are barely detectable around the perinatal period, but increase gradually after birth (D'ercole and Underwood, 1980; Tiong and Herington, 1992). Hepatic *IGF1* also increases between 10 and

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20 days post-partum and circulating IGFALS concentrations at day 30 post-partum (Frystyk et al., 1998; Tiong and Herington, 1992).

Marsupials have a different reproductive strategy. Even in the largest species, the young are minute at birth (<0.01% of maternal weight and maximum of 1 g) and are developmentally equivalent to an 8–9 week old human embryo. In contrast to eutherian species, in marsupials the increase in hepatic *GHR* expression and plasma IGF-I concentrations associated with growth axis maturation occurs many months after birth during pouch life, but at an equivalent developmental stage to that of most eutherians (Menzies et al., 2012; Saunders et al., 2003).

In eutherian mammals, it has been hypothesized that growth axis maturation occurs during the transition from fetal to post-natal life, so that the mother can restrain fetal growth in the uterus via nutrition to prevent fetal overgrowth (Gluckman and Hanson, 2004). However, given that growth axis maturation occurs after birth in rodents, humans and marsupials it would appear that it is only coincidentally coordinated with birth in species like the sheep (Li et al., 1996). Thus, maturation of the endocrine growth axis appears to be a developmental event that has no direct functional link to the timing of birth.

Tammar wallaby (*Macropus eugenii*) pouch young can be transferred at day 60 of pouch life to mothers at advanced lactational stages, dramatically accelerating their growth and developmental rate (Trott et al., 2003). The effects are thought to be due to changes in the provision of essential nutrients by the mother to the young. Milk production and composition of macronutrients (protein, carbohydrate and lipid) change markedly through the long lactation period of the tammar wallaby (Green et al., 1983, 1988; Green and Renfree, 1982; Joss et al., 2009; Messer and Green, 1979; Messer and Nicholas, 1991; Messer et al., 1984; Nicholas, 1988). Consequently, young 'fostered forward' have access to a higher volume of richer milk and an earlier transition to the high fat, high energy, complex protein milk relative to age-matched controls (Kwek et al., 2009; Trott et al., 2003; Waite et al., 2005).

Lambs receiving a high nutritional diet during fetal and early post-natal life have increased hepatic mRNA expression of *GHR*, *IGF1* and *ALS* relative to lambs receiving a poor diet, suggesting that lambs on a richer diet may progress through GH-IGF axis maturation earlier (Rhoads et al., 2000b). Altogether, the data suggest that the maturation of the growth axis may be a pliable developmental event that does not depend on birth but rather on nutritional status and maturity of the young.

To determine whether the maturation of the growth axis is affected by nutrition, growth and developmental rate, we fostered tammar wallaby pouch young at day 60 after birth to foster mothers from which we removed their 120 day old young. We euthanized the fostered young 60 and 90 days later at days 120 and 150 of development and compared their weight, developmental milestones, plasma GH and IGF-I concentrations, pituitary *GH* mRNA expression, and liver *GHR*, *IGF1* and *2*, *IGFBP-2* and *-3*, and *IGFALS* mRNA expression with that of age-matched controls and the slow growing young of primiparous mothers in their first lactational cycle.

2. Materials and methods

2.1. Animals

Tammar wallabies (*Macropus eugenii*) of Kangaroo Island (South Australia) origin were maintained at the marsupial research facility of The University of Melbourne (Victoria, Australia). All animals were collected with the approval of the South Australian Department of Environment and Natural Resources and colony animals were held under permits from the Victorian Department of Sustainability and Environment. All experiments were approved by the University of Melbourne Institutional Animal Ethics Committee and in accordance with the National Health and Medical Research Council (2013) guidelines. The diet of the animals consisted of pasture supplemented with lucerne cubes and water provided *ad libitum*.

Table 1
Total animals and mortality rate in each experimental group.

	Slow	Normal	Fast
Total	25	43	28
Euthanized 120 days	7	16	7
Euthanized 150 days	0	12	4
Survived to first pouch exit (~200 days)	3	8	1
Lost from pouch after first transfer	15	7	16
Lost from pouch at 120 days			
Lost from pouch by 150 days			
Mother died so pouch young lost			
Random loss			
Mortality rate of young %	60	16	57

The young were sexed from external morphology and age was assessed using head length tables (Poole et al., 1991). Females with pouch young were assigned into one of three groups on the basis of their body weight. For the *normal* and the *fast* growth (i.e. foster forward) groups, adult females of an average of 5.2 ± 0.5 kg and 5.13 ± 0.21 kg respectively were used to avoid any influences that initial maternal weight may have on the weight of the young (Green et al., 1988). Primiparous mothers in their first year of reproduction and between 2.6 and 3.8 kg (average 3.08 ± 0.4 kg) were assigned to the *slow* growth group. Females in this category have slower growing young, and many lose their young in later stages of lactation (Nurse and Renfree, 1994; Williams et al., 1998). In the age range of this study there is no significant difference in growth rate of male and female young (Poole et al., 1991). For the *fast* growth group, 60-day-old young were removed from their mother's pouch and placed in the pouch of mothers carrying young at 120 days of lactation. The 120-day pouch young of these mothers were removed and euthanized so their tissues could be used as additional controls (*normal*) for later comparisons at day 120 post-partum.

Young from each group were removed from the pouch every 15 days to measure body weight, developmental milestones including whisker development, opening of eyes, motor coordination and first appearance of velvet fur. When they reached 120 days of age, pouch young were euthanized using pentobarbitone sodium (60 mg/mL i.p.; 150 mg/kg). A second group of young from each group was kept for a further 30 days (150 days old) and then euthanized, and a third group was maintained alive until first pouch exit (≈200 days) (numbers of animals are detailed in Table 1). Survival of young from primiparous females is poor (Williams et al., 1998), so there were too few young surviving at 150 days of age to compare the *slow* growth group with the other two groups statistically. Young were also lost from the *fast* group. Many of these young were lost at the first transfer (Table 1). Manually reattaching them to the teat can be stressful for the mother, so young were placed into their foster mother's pouch. These young were absent from the pouch at the first post-transfer capture, so were presumed lost due to a failure to re-attach to the larger teat. A few mothers died during the experiment, especially during one winter period with an exceptionally high rainfall. The remaining losses occurred randomly (e.g. mother dropping the young from the pouch; mother kicked pouch young). These details are summarized in Table 1.

2.2. Tissue collection

Organs including pituitary, brain, liver, kidney and heart were removed from each animal and weighed separately. Small samples of livers (right lobe) were transferred to cryotubes, immersed in