

Development of the diaphragm and the effects of maternal creatine supplementation on birth hypoxia in a novel precocial species, the spiny mouse (*Acomys cahirinus*)

D. Cannata,¹ Z. Ireland,² H. Dickinson,³ D.W. Walker² and J.M. West,¹ ¹School of Life and Environmental Sciences, Deakin University, 221 Burwood Hwy, Burwood, VIC 3125, Australia, ²Department of Physiology, Monash University, Clayton, VIC 3168, Australia and ³Monash Immunology & Stem Cell Laboratories, Monash University, Clayton, VIC 3168, Australia.

The diaphragm is the main respiratory muscle that enables us to breathe. It is the only skeletal muscle that must function at the onset of birth. Even though the importance of the diaphragm is well established, little is known about the transition the diaphragm undergoes when the lungs are filled with embryonic fluid to when they are filled with air when breathing is established. Breathing difficulties are common amongst newborns, particularly in cases of pre-mature birth or when development has been compromised by hypoxia. Regardless of the origin of these difficulties, in these conditions the demand on the diaphragm is high, thus it is conceivable that respiratory failure can be due to the diaphragm being unable to meet demands of the respiratory system.

Skeletal muscle fibres are generally categorised as either slow- or fast-twitch, distinguishable by their contractile and activation properties (West & Stephenson, 1993), and expression of unique proteins and ATPase isoforms. In some species (e.g. sheep), the diaphragm is composed of 'hybrid' fibres that express both slow- and fast-twitch ATPase isoforms and exhibit activation properties characteristic of both fibre types. The aim of this study was to examine the characteristics of diaphragm muscle fibres before and after birth as well as the effect of hypoxia in a small precocial animal, the spiny mouse (*Acomys cahirinus*). In addition, maternal creatine supplementation was administered to determine if creatine resulted in an increase survival rate of neonates subjected to birth hypoxia.

Diaphragm muscle was collected from fetal, newborn and adult spiny mice, pinned onto dental wax at an approximate *in vivo* length, and stored in a relaxing solution containing glycerol at -20°C until use. Single fibres were then isolated by dissection, chemically 'skinned' (membrane removed), and Ca²⁺- and Sr²⁺-activation profiles obtained. Samples were also snap frozen and stored at -80°C to determine myosin ATPase, NADH⁺ and succinate dehydrogenase activity. For the hypoxia model, neonates were placed under hypoxic conditions for 8 minutes before they were able to take their first breath at birth. Samples were collected 24 hours after birth as described above. Half the pregnant dams were given a 5% creatine diet during their pregnancy.

In the diaphragm of fetal spiny mouse, the Ca²⁺- and Sr²⁺-activation profiles were characteristic of a fast-twitch fibre (large difference in sensitivity to Ca²⁺ and Sr²⁺). However, a transition occurs in the last few days of gestation providing evidence that slow-twitch isoforms are activated. There is an increased sensitivity to Sr²⁺. (pCa₁₀-pSr₁₀ value decreasing by 0.28 units). ATPase and MHC expression also showed a similar transition at this time with the number of Type I (slow-twitch) fibres doubling (6% to 12%), and the presence of both I and IIa MHC isoforms (oxidative isoforms). Thus considerable re-modelling of the diaphragm occurs just before birth. This suggests that the metabolic characteristics of the diaphragm shift to become more 'oxidative/fatigue resistant'. In male animals, the effect of hypoxia decreased the survival rate (20%), however the survival rate increased with creatine supplementation (80%). In these conditions creatine may provide extra structural support to the diaphragm. Cross-sectional area (CSA) of diaphragm fibres in newborns decreased under hypoxic conditions (510µm² ± 28.6), unless the mother was supplemented with creatine, to which CSA was normal (950µm² ± 30.4). The survival rate of female animals that underwent hypoxia, remained relatively high (80%). Creatine supplementation did not increase the survival rate (80%) of the female newborns.

West JM, Stephenson DG (1993) *Journal of Physiology*, **462**: 579-596