Developmental changes in contractile function of the diaphragm in the pre-term lamb

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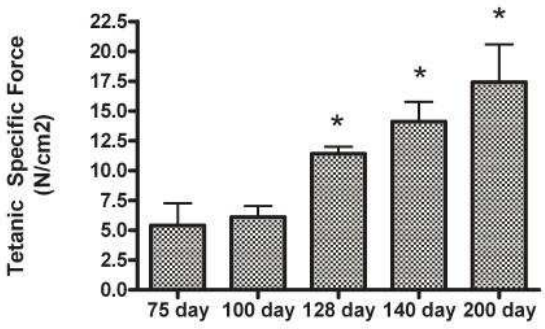
The reduced force producing capacity and decreased fatigue resistance that arise from mechanical ventilation have been termed ventilator induced diaphragm dysfunction (VIDD). Although VIDD has been characterised in adults after mechanical ventilation, no study has systematically explored the existence of and pre-disposing factors to VIDD in the newborn subject. We propose that the functional development of the diaphragm may play a role in the onset of VIDD in neonates as gestational differences in transdiaphragmatic pressure and maximal inspiratory pressure have been observed (Dimitriou *et al.,* 2001). Developmental changes in the contractile function of the diaphragm have been examined primarily in small laboratory animals. However, most developmental changes in rodents occur *ex utero* while the majority of development in human neonates occurs *in utero* (Finkelstein *et al.,* 1992). Furthermore, the higher proportion of fast fibres and higher respiratory rate in small laboratory animals (Hodge *et al.,* 1997) may impact on the contractile properties of the diaphragm and therefore affect the susceptibility to damage, fatigue and VIDD. As little is known about the *in utero* development of fibre type composition and contractile properties of the diaphragm in human neonates, we used a comparable ovine animal model to investigate developmental changes in contractile function of the diaphragm at different gestational ages.

Fetuses of different ages were delivered at 75 days (n = 4), 100 days (n = 4), 128 days (n = 6) or 140 ± 2 days (n = 4) gestation or delivered at term (~150 days) and grown to 7 weeks postnatal age (200 day group; n = 4). The fetal diaphragm was removed after euthanasia via a lethal umbilical venous injection of pentobarbitone (100 mg/kg) and exsanguination via the femoral artery. Two strips of longitudinally arranged muscle fibres were dissected from the diaphragm muscle to assess 1) the susceptibility to muscle damage and 2) the recovery after fatigue. The rib and tendon ends of the muscle strip were tied with surgical silk and the preparation mounted in an *in vitro* muscle test system (1200A, Aurora Scientific Inc.) perfused with mammalian saline solution (in mM): NaCl (109), KCl (5), $MgCl_2$ (1), $CaCl_2$ (4), $NaHCO_3$ (24), NaH_2PO_4 (1), sodium pyruvate (10) and aerated with 95% O_2-5% CO_2 at 25°C. The contractile parameters were determined including maximum isometric tetanus (P_0), specific tetanic force (force/cross-sectional area, Sp_o), force-frequency relationship, maximum isomeric twitch force (P_t), time to P_t, half relaxation time, maximum rate of force development (dF/dt) before and after either a fatigue protocol (150 × 330 ms tetani trains, 80 Hz) or muscle damage protocol (5 × stretches at 10% fibre length during isometric plateau phase of Po). Fatigue index was calculated by dividing the 150th tetanic stimulus by the 1st tetanic stimulus during the fatigue protocol.

A number of significant (one-way ANOVA, bonferroni post-hoc, $p < 0.05$) age-dependant changes were seen, including: 1) three-fold increase in specific tetanic force from 75 d to 200 d (see Figure); 2) force frequency relationship increased by a factor of ~3 from 75 d to 200 d; 3) fatigue index decreased by half from 75 d to 200 d; 4) mean time to recovery after fatigue increased from 5 min at 75 d to 40 min at 200 d; 5) dF/dt decreased from 700 (g/s) at 100 d to 100 (g/s) at 200 d.



Mean and S.E. *p<0.05 compared with 75 day and 100 day groups

This study shows age-dependant changes in contractile function of the sheep diaphragm including increased Sp_0 and decreased fatigue resistance with advancing maturation. These changes may reflect a shift in fibre type composition from slow to fast fibres in the maturing diaphragm, or differences in the contractile function of fetal and neonatal muscle fibres. These data provide a framework from which we can investigate our hypothesis that the magnitude and nature of VIDD is influenced by gestation.

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