Impact of antenatal inflammation on diaphragm muscle function in the preterm lamb

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The incidence of respiratory failure is higher in preterm babies than at any other time of life, and a functional diaphragm is pivotal to successful establishment of unsupported spontaneous breathing. Therefore, the integrity of the diaphragm at delivery may critically influence the susceptibility to respiratory failure. Clinically relevant antenatal exposures such as inflammation and sepsis may affect the development and function of the fetal diaphragm. *In utero* exposure to inflammation is common in the preterm infant and chorioamnionitis (inflammation of the placental/fetal membranes) is implicated in up to 70% of preterm births (Cheah *et al.*, 2008). A 7 d *in utero* exposure to intra-amniotic LPS increases circulating neutrophils (Kramer *et al.*, 2001), causes systemic oxidative stress (Cheah *et al.*, 2008) and is likely to contribute to contractile dysfunction in skeletal muscle. Therefore, we hypothesize that the preterm diaphragm is susceptible to phenotypic alteration induced by antenatal inflammation.

We used an established newborn sheep model of neonatal lung disease to determine the impact of *in utero* inflammatory exposure on the contractile function of the newborn diaphragm. Experiments were conducted according to the NHMRC guidelines for the use of animals in experimental research and approval was obtained from the Animal Ethics Committee at the University of Western Australia. Pregnant ewes (n=14) received ultrasound guided intra-amniotic injections of saline or LPS (10 mg, *E. coli* O55:B5; Sigma, Melb, Aust) 2 d or 7 d prior to delivery. Ewes were sedated (IV Ketamine 10 mg/kg, Medetomidine 0.02 mg/kg) and received a spinal anaesthetic (2% Lignocaine, 3 mL) prior to the premature delivery of lambs at 121 d gestation (term = 150 d). Cord arterial blood gases, plasma and complete blood count were obtained prior to euthanasia of the fetus (100 mg/kg pentobarbitone and exsanguination). At post-mortem, the right hemidiaphragm was removed for assessment of contractile function. Longitudinal strips of diaphragm muscle fibres were mounted in an *in vitro* muscle test system (1200A, Aurora Scientific Inc. Ontario, Canada) containing physiological saline solution (in mM: NaCl, 109; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 4; $NaHCO_3$, 24; NaH_2PO_4, 1; sodium pyruvate, 10), at 25 °C and bubbled with carbogen. The muscle was adjusted for optimum muscle length (L_0) and isometric twitch and tetanic contractions were recorded. Functional measurements to determine susceptibility to fatigue (measured by the force deficit following 3 min intermittent contraction using 1 s tetanic stimulation at 80 Hz every 5 s) and stretch-induced muscle damage (force deficit following 5 eccentric contractions of 10% L_0) were performed. Finally, muscle mass and fibre length were recorded for assessment of specific force relative to fibre cross-sectional area.

Acute *in utero* exposure to an inflammatory stimulus caused significant (~30%) weakness in the diaphragm. Maximum diaphragmatic specific forces after 2 d (11.2 ± 1.3 N.cm^{-2}) and 7 d (12.7 ± 1.0 N.cm^{-2}) exposure to LPS were significantly lower than saline controls (16.7 ± 1.4 N.cm^{-2}; $P<0.05$). Similarly, the peak twitch forces after 2 d (5.7 ± 0.8 N.cm^{-2}) and 7 d (6.5 ± 0.3 N.cm^{-2}) LPS exposure were significantly lower than that recorded after saline exposure (8.4 ± 0.9 N.cm^{-2}; $P<0.05$). After 2 d LPS exposure, the twitch half-relaxation time (22.0 ± 1.7 ms) was significantly longer than both 7 d LPS (18.2 ± 1.0) and saline controls (18.0 ± 1.1 ms; $P<0.05$). There were no significant differences in the susceptibility to fatigue or to stretch-induced muscle damage between the treatment groups.

These data indicate that both short term (2 d) and longer term (7 d) *in utero* exposure to inflammation causes contractile dysfunction and muscle weakness in the pre-term diaphragm. Therefore, inflammatory conditions such as chorioamnionitis are likely to compromise the integrity of the diaphragm at delivery and may critically influence the resilience of the infant to developing respiratory failure after birth.

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Kramer BW, Moss TJ, Willet KE, Newnham JP, Sly PD, Kallapur SG, Ikegami M, Jobe AH. (2001) *American Journal of Respiratory and Critical Care Medicine,* **164(6):** 982-988.