The mechanism(s) of vitamin D deficiency induced alterations in lung structure and function

G.R. Zosky, E. Bennett, N. Nuñez and L. Chen, School of Medicine, Faculty of Health, University of Tasmania, TAS 7000, Australia.

Background: There are a plethora of studies showing cross-sectional associations between vitamin D deficiency and chronic lung diseases including asthma and chronic obstructive pulmonary disease (Foong and Zosky, 2013). However, these associations are confounded by the effect of chronic disease on physical activity levels (Strine *et al*., 2007) which are highly correlated with sun exposure and, hence, vitamin D synthesis (Scragg & Camargo, 2008). In line with this, randomised controlled trials using vitamin D supplementation to ameliorate chronic lung disease have been disappointing. In contrast, we have strong longitudinal evidence to suggest that vitamin D deficiency has a detrimental impact on lung development (Foong *et al.*, 2015; Zosky *et al.*, 2011, 2014). In this study we aimed to identify the potential mechanisms linking vitamin D deficiency with altered lung development using a mouse model.

Methods: We established colonies of vitamin D deficient and replete female BALB/c mice, using dietary manipulation (Zosky *et al.*, 2011), that were mated with replete male mice. Mice were euthanased by i.p. ketamine/xylazine overdose (800/40 mg/kg) at key developmental timepoints (E14.5, E17.5 or P7) and lung tissue was collected for proteomic analysis by liquid chromatography-mass spectrometry (LCMS). Differentially expressed proteins were identified using a 0.05 false discovery rate (FDR) with the expression of key proteins with a biologically plausible link with lung function confirmed by enzyme-linked immunosorbent assay (ELISA) in separate cohorts of mice. Separate groups of mice had their lungs fixed in formalin for histological assessment of lung structure by stereology.

Results: There was no difference in protein expression between vitamin D deficient and replete mice at the E14.5 and E17.5 timepoints. Proteomic analyses identified 39 proteins that were differentially expressed in the lungs of P7 vitamin D deficient mice compared to P7 vitamin D replete mice. ELISA confirmed the reduced expression of surfactant protein B (Sftpb) in the lungs of vitamin D deficient P7 mice compared to replete P7 mice (P=0.002). There was no difference in the expression of collagen type I a1 (col1A1), a second protein with a plausible link to lung function, as assessed by ELISA. There also was no difference in lung volume (P=0.60), volume of the airspaces (P=0.67) or surface area (P=0.76) between the lungs of P7 vitamin D deficient and replete mice.

Discussion: The observed differences in protein expression suggest that vitamin D deficiency induced alterations in lung structure and function occur during alveolarisation and are driven by altered surfactant synthesis. The lack of difference in lung structure at P7, in contrast to existing data showing structural alterations at P14 (Zosky *et al.*, 2011), suggest that the effect of early life vitamin D deficiency on lung function is not realised until later in alveolar development. These data provided a plausible mechanism linking maternal vitamin D deficiency with altered post-natal lung function.

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