

Vitamin D Deficiency Causes Deficits in Lung Function and Alters Lung Structure

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Rationale: The prevalence of vitamin D deficiency is increasing and has been linked to obstructive lung diseases including asthma and chronic obstructive pulmonary disease. Recent studies suggest that vitamin D deficiency is associated with reduced lung function. The relationship between vitamin D deficiency and lung function is confounded by the association between physical activity levels and vitamin D status. Thus, causal data confirming a relationship between vitamin D and lung function are lacking.

Objectives: To determine if vitamin D deficiency alters lung structure and function.

Methods: A physiologically relevant BALB/c mouse model of vitamin D deficiency was developed by dietary manipulation. Offspring from deficient and replete colonies of mice were studied for somatic growth, lung function, and lung structure at 2 weeks of age.

Measurements and Main Results: Lung volume and function were measured by plethysmography and the forced oscillation technique, respectively. Lung structure was assessed histologically. Vitamin D deficiency did not alter somatic growth but decreased lung volume. There were corresponding deficits in lung function that could not be entirely explained by lung volume. The volume dependence of lung mechanics was altered by deficiency suggesting altered tissue structure. However, the primary histologic difference between groups was lung size rather than an alteration in architecture.

Conclusions: Vitamin D deficiency causes deficits in lung function that are primarily explained by differences in lung volume. This study is the first to provide direct mechanistic evidence linking vitamin D deficiency and lung development, which may explain the association between obstructive lung disease and vitamin D status.

Keywords: vitamin D; lung function; lung structure; mouse model

There has been a dramatic increase in the prevalence of vitamin D deficiency around the world (1, 2). Vitamin D deficiency is associated with a number of diseases, in particular the bone disorder rickets as a result of the role of vitamin D in calcium homeostasis (3). However, the active form of vitamin D ($1\alpha,25[\text{OH}]_2\text{D}$) is also critical in immune regulation (4), and deficiency of this vitamin has been linked to both autoimmune disease (5) and cardiovascular disease (6). In addition, the vitamin D axis has been implicated in the pathogenesis of chronic respiratory diseases including asthma (7, 8) and chronic obstructive pulmonary disease (COPD) (9–11).

Epidemiologic studies have shown an association between (1) low maternal vitamin D intake and wheeze in children (12–14), (2) decreased serum levels of vitamin D and increased asthma severity (15) and steroid use (16) in children with asthma,

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

The prevalence of vitamin D deficiency is increasing and has been associated with obstructive lung disease. There is an association between vitamin D deficiency and lung function, which may explain this link.

What This Study Adds to the Field

This study provides direct evidence for a causal link between vitamin D deficiency and deficits in lung function and altered lung structure.

and (3) reduced glucocorticoid responses in adults with asthma with low serum vitamin D (17). A similar association exists between COPD severity and low levels of serum vitamin D (10). However, it has been demonstrated that low serum vitamin D levels are associated with physical inactivity (18–20). Thus, given the known association between increased asthma (21) and COPD (22) severity and low physical activity levels, a causal link between vitamin D and these respiratory diseases has been difficult to establish.

Given the immunomodulatory properties of vitamin D (23), previous studies have primarily focused on immune mechanisms of lung disease. However, vitamin D may also play a role in lung development, which could explain the association between vitamin D deficiency and lung disease in the absence of alterations in immune regulation. For example, data from the third United States National Health and Nutrition Examination Survey showed a strong relationship between serum vitamin D and baseline lung function (FEV_1 and FVC) (24). This association between vitamin D levels and lung function is also seen in COPD (10). Similarly, vitamin D increases surfactant synthesis (25), inhibits airway smooth muscle proliferation (26), and has a critical role in epithelial-mesenchymal interactions during lung growth (25). However, there has been no study to determine directly whether vitamin D deficiency alone results in altered lung function *in vivo*. Additionally, the effect of vitamin D deficiency *in utero* on fetal growth is controversial and seems to be dependent on maternal calcium status (27). There is a well-known relationship between body size and lung function, so any effect of vitamin D on somatic growth ultimately influences lung function in the absence of a direct effect on the lung. The nature of the cross-sectional population-based studies that have shown an apparent relationship between vitamin D deficiency and lung function means that a causal relationship between vitamin D deficiency alone, without additional confounders, and altered lung growth resulting in altered lung function is yet to be established.

To date there is only limited mechanistic evidence for a direct role for vitamin D in the progression of obstructive respiratory

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disease, which can be partially explained by the limited utility of experimental mouse models of altered vitamin D regulation. This is caused by the extreme phenotype of both the 1α -hydroxylase (28) and vitamin D receptor (29) knockout mouse models, which both develop severe hypocalcaemia (and the associated bone malformations) and hyperparathyroidism. To overcome this problem we have developed a physiologically relevant mouse model of vitamin D deficiency with serum levels of vitamin D matching those seen in deficient human populations.

The aim of this study was to determine if vitamin D deficiency results in altered lung function or structure as a potential explanation for the association between vitamin D and chronic respiratory disease. Specifically, we aimed to determine if vitamin D deficiency (1) has an influence on somatic growth, (2) results in delayed lung growth as indicated by a decrease in lung volume after controlling for changes in somatic growth, (3) alters the mechanical properties of the lung tissue as indicated by the volume dependence of lung mechanics, and (4) results in alterations in lung morphology.

METHODS

Model

Three-week-old female BALB/c mice (ARC, Murdoch, Western Australia) were provided with vitamin D-deficient or -replete (2,195 IU/kg) diets (Specialty Feeds, Glen Forrest, Western Australia) for at least 5 weeks before mating. In all cases, female mice on the vitamin D-deficient diets were confirmed as being deficient (by assay of serum vitamin D levels) before mating at 8 weeks of age. Deficient diets were supplemented with calcium 25 g/kg (vs. 15 g/kg) to avoid hypocalcaemia and the caloric content of the diets was adjusted to ensure that all mice had similar calorie intake (deficient, 15.3 MJ/kg; replete, 15.8 MJ/kg). Mice were housed in rooms with a 12:12 hour ambient ultraviolet B free light-dark cycle. Food and water were provided *ad libitum*. Female mice were mated with vitamin D-replete males and offspring of both sexes were studied at 2 weeks of age for somatic growth, lung volume, lung function, and lung structure. All studies were performed according to animal health and welfare guidelines and were approved by the Institutional Animal Ethics Committee.

Mechanical Ventilation

Mice were anesthetized by intraperitoneal injection with ketamine (20 mg/ml; Troy Laboratories, NSW, Australia) and xylazine (1 mg/ml; Troy Laboratories) at a dose of 0.01 ml/g. Two-thirds of the dose was given before tracheostomy and cannulation. The remaining anesthetic was given and mice were placed in a plethysmograph and mechanically ventilated (HSE-Harvard MiniVent; Harvard Apparatus, Holliston, MA) at 400 breaths/min with a tidal volume of 10 ml/kg and 2 cm H₂O positive end-expiratory pressure.

Lung Volume

Thoracic gas volume (TGV) was measured as described previously (30). The trachea was occluded at elastic-equilibrium lung volume (EELV) and inspiratory efforts were induced by intramuscular electrical stimulation. TGV was calculated by applying Boyle's law to the tracheal and box pressure signals (30).

Lung Mechanics

Lung mechanics were assessed using a modified low-frequency forced oscillation technique (31). Briefly, a speaker generated an oscillatory signal containing nine frequencies ranging from 4–38 Hz. The signal was delivered to the tracheal cannula via a wavetube of known impedance. A model with constant phase tissue impedance was fit to the respiratory impedance spectrum (Z_{rs}) allowing calculation of the Newtonian resistance (R_{aw} ; which approximates airway resistance in mice); airway inertance (I_{aw} ; which is negligible after correcting for the tracheal cannula); tissue damping (G); and tissue elastance (H).

Hysteresivity (η) was calculated by G/H (32). This system allowed assessment of the volume dependence of lung mechanics (31).

Lung Structure

Lung structure was assessed according to American Thoracic Society and European Respiratory Society guidelines (33). After euthanasia the tracheal cannula was instilled with 2.5% glutaraldehyde at 10 cm H₂O. This fixation pressure was chosen to fall within the range of volumes that lung function was measured at EELV (34). Lungs were randomly oriented (35) and embedded in paraffin. Starting at a random point, sections (5 μ m) were taken at regular (500 μ m) intervals throughout the lung and stained with hematoxylin and eosin. Lung volume (V_L) was calculated using the Cavalieri method (36) and point counts were used to obtain total tissue volume (V_t); volume of the alveolar septa (V_s); and air in the major airways (V_a), alveolar ducts, (V_{ad}) and alveoli (V_{alv}). Alveolar surface area (S_a) was calculated using a linear grid and S_a and V_s were used to estimate the mean (arithmetic) septal thickness (T_s) (33). The depth-diameter ratio of the alveoli was also calculated by direct measurement (37) as an index of alveolar septation. Alveolar number (N_a) was calculated using a physical disector (38).

Statistics

Between-group comparisons were made using *t* tests. Additional analyses involving correction for continuous variables (e.g., body size and lung volume) were conducted using analysis of covariance. Data were analyzed in Stata (v11; StataCorp, College Station, TX) and reported as mean (SD).

RESULTS

Model Characteristics

Vitamin D deficiency had no effect on litter size (deficient 4.9 [2.8] vs. replete 3.9 [2.1]; $P = 0.39$). Thirty-four replete (female, $n = 13$; male, $n = 21$) and 47 deficient (female, $n = 25$; male, $n = 22$) offspring were studied for somatic growth and lung function. Serum vitamin D levels in the deficient mice (12.8 [2.3] nmol/L) were significantly lower than those in the replete mice (81.5 [27.9] nmol/L) and below that of the consensus cutoff value for deficiency in humans of 50 nmol/L (3). There was no difference in serum calcium (Ca^{2+}) levels between the two groups (deficient 8.90 [3.87] mg/dl vs. replete 9.05 [2.45] mg/dl; $P = 0.94$) and no evidence for a difference in the percentage bone mineral content per body weight between the groups as measured by dual-energy X-ray absorptiometry (GE Lunar Prodigy; GE Lunar Corporation, Madison, WI) (deficient 9.1 [0.7]% vs. replete 9.1 [1.2]%; $P = 0.97$).

Somatic Growth

There was no evidence for a difference in body weight between mice born to vitamin D-deficient or -replete mothers (female, $P = 0.34$; male, $P = 0.40$) (Figure 1). There was some evidence to suggest that male vitamin D mice were significantly shorter ($P = 0.04$) than their replete counterparts. However, this was not the case in females ($P = 0.42$) (Figure 1) and the magnitude of the difference in the males was small (~ 2 mm or 3.5%).

TGV

Both male ($P < 0.001$) and female ($P = 0.001$) vitamin D-deficient mice had significantly smaller TGV than replete controls (Figure 2). These differences were still apparent after correcting for body length (male, $P < 0.001$; female, $P = 0.001$) (Figure 2).

Baseline Lung Mechanics

R_{aw} and tissue mechanics (G and H) were significantly higher in both male (R_{aw} , $P < 0.001$; G, $P < 0.001$; H, $P < 0.001$) and female (R_{aw} , $P < 0.001$; G, $P = 0.004$; H, $P = 0.03$) vitamin D-deficient mice compared with their respective replete controls

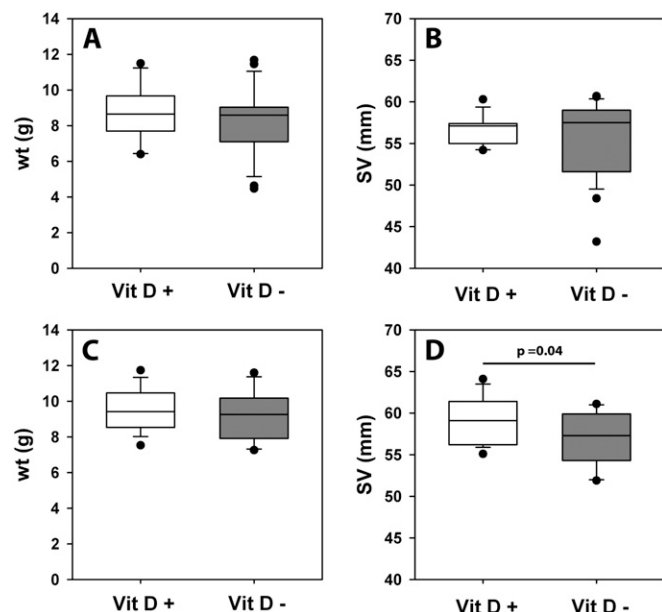


Figure 1. Box plots (median, interquartile range, and range) of weight (wt) and snout vent (SV) length for female (A, B) and male (C, D) vitamin D-replete (white) and -deficient (gray) mice at 2 weeks of age.

(Figures 3 and 4; data not shown for G). There was no difference in hysteresivity (a fundamental property of the lung tissue describing the ratio of energy dissipation to energy storage) between deficient and replete mice for either sex (male, $P = 0.53$; female, $P = 0.29$; data not shown). For males these differences were still evident in R_{aw} ($P = 0.001$) and H ($P = 0.04$), but not G ($P = 0.10$), after correcting for lung volume. In contrast, only R_{aw} was significantly higher in females after correcting for lung volume ($P = 0.001$), whereas this was not the case for G ($P = 0.15$) or H ($P = 0.40$) suggesting that differences in tissue mechanics at baseline in female vitamin D-deficient mice could be explained by differences in lung volume (Figures 3 and 4; data not shown for G).

Volume-dependent Lung Mechanics

Because of the differences in lung volume at baseline, the pressure-volume curve in male and female vitamin D-deficient mice was shifted downward. There was a corresponding difference in the lung volume reached at 20 cm H₂O transrespiratory pressure (P_{rs}) in male ($P < 0.001$) and female ($P < 0.001$) mice. However, this difference seemed to be proportional to lung volume at baseline in both sexes (male, $P = 0.07$; female, $P = 0.44$) (Figure 5). At 20 cm H₂O R_{aw} (male, $P = 0.003$; female, $P = 0.01$), G (male, $P = 0.002$; female, $P = 0.002$), and H (male, $P < 0.001$; female, $P < 0.001$) were all higher in the vitamin D-deficient mice compared with replete mice (Figure 6; data not shown for G). For R_{aw} this could be explained by the difference in lung volume at 20 cm H₂O because all values for R_{aw} seemed to fall on a master curve describing the relationships with lung volume, whereas this was not the case for G or H where the relationship between these parameters and lung volume in vitamin D-deficient mice clearly deviated substantially from that observed in the vitamin D-replete mice.

Lung Structure

Postfixation and embedding lung volume was significantly smaller in vitamin D-deficient mice (female, $n = 8$; male, $n = 6$) compared with replete mice (female, $n = 8$; male, $n = 7$) ($P = 0.05$) with no effect of sex ($P = 0.92$) (Figure 7). There was no difference in the volume of the major airways (V_a) between the groups ($P = 0.64$). However, the volume of air in the alveolar ducts (V_{ad}) was significantly lower in the vitamin D-deficient mice ($P = 0.02$) with no effect of sex ($P = 0.25$) (Figure 7). There was some evidence to suggest that the volume of tissue in the alveolar septa ($P = 0.08$) was lower in vitamin D-deficient mice compared with the replete mice; however, there was no difference in either surface area ($P = 0.27$) or septal thickness ($P = 0.55$) between groups (data not shown). The total tissue volume (V_t) was significantly lower ($P = 0.01$) in the vitamin D-deficient mice (female, vit D⁺ 0.074 [0.009] ml vs. vit D⁻ 0.062 [0.010] ml; male, vit D⁺ 0.070 [0.007] ml vs. vit D⁻ 0.059 [0.016] ml) with no effect of sex ($P = 0.44$). In contrast, there was no difference in the depth to diameter ratio of the alveoli ($P =$

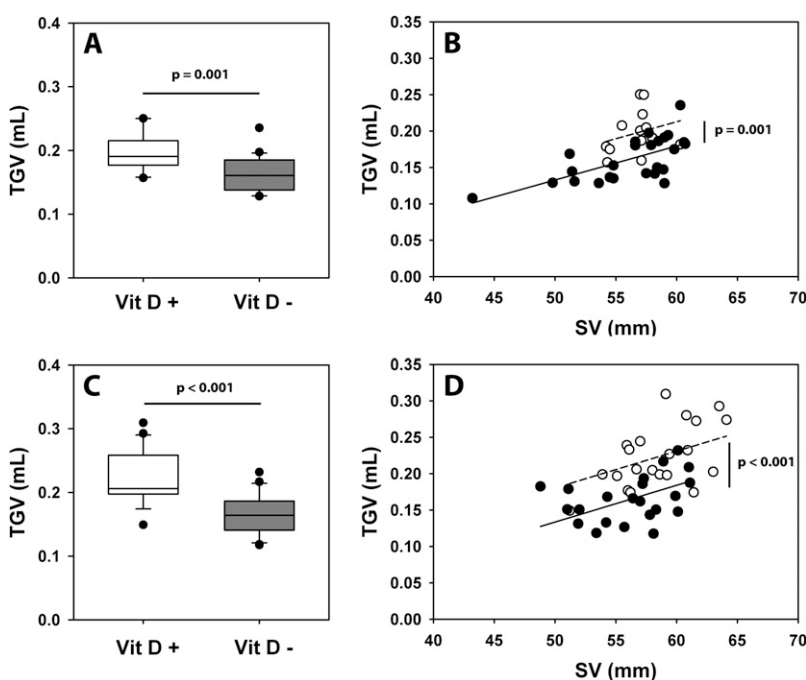


Figure 2. Box plots (median, interquartile range, and range) of thoracic gas volume (TGV) and scatter plots of TGV against snout vent (SV) length with regression lines from analysis of covariance for female (A, B) and male (C, D) vitamin D-replete (white symbols, dashed lines) and -deficient (dark symbols, solid lines) mice at 2 weeks of age.

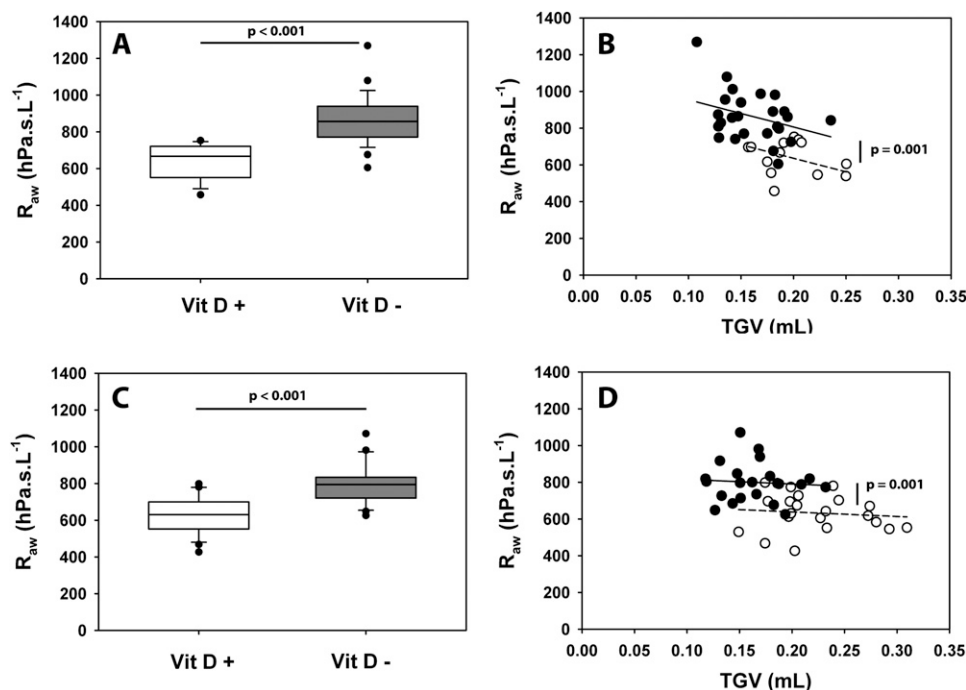


Figure 3. Box plots (median, interquartile range, and range) of airway resistance (R_{aw}) and scatter plots of R_{aw} against thoracic gas volume (TGV) with regression lines from analysis of covariance for female (A, B) and male (C, D) vitamin D-replete (white symbols, dashed lines) and -deficient (dark symbols, solid lines) mice at 2 weeks of age.

0.92) between the groups (female, vit D⁺ 0.890 [0.013] vs. vit D⁻ 0.871 [0.029]; male, vit D⁺ 0.877 [0.013] vs. vit D⁻ 0.892 [0.025]). The number of alveoli in vitamin D-deficient female mice was lower than that in replete females ($P = 0.06$), whereas this was not the case in male mice ($P = 0.97$) (Figure 8). Despite this, there was no difference in the arithmetic mean volume of the alveoli between groups of either sex (female, $P = 0.18$; male, $P = 0.20$) (data not shown).

DISCUSSION

This study clearly demonstrated that vitamin D deficiency causes decrements in lung function. These differences could

not be attributed to alterations in somatic growth and seemed to be over and above the influence of differences in lung volume. These deficits in lung function were reflected histologically and related primarily to differences in overall lung size.

For the first time direct mechanistic evidence has been provided supporting a relationship between vitamin D and lung growth *in vivo* whereby vitamin D deficiency resulted in a significant deficit in lung volume. It is well known that there is a strong association between body size and lung volume (39). Importantly, although there was a small but statistically significant difference in body length between male vitamin D-deficient and -replete mice, vitamin D did not seem to have a large impact on somatic growth. Correspondingly, differences we observed in

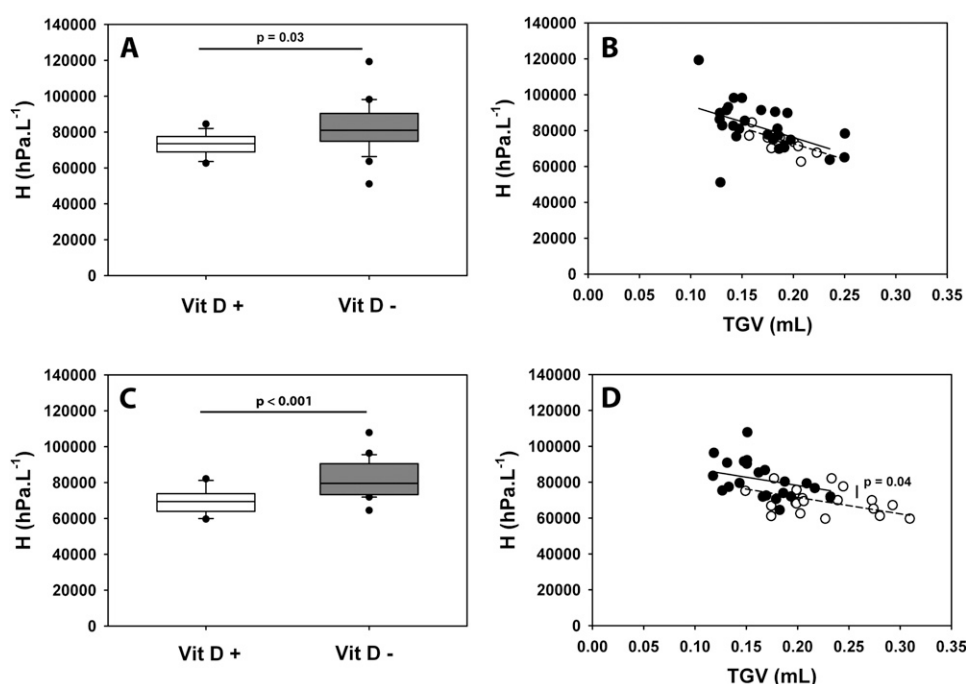


Figure 4. Box plots (median, interquartile range, and range) of tissue elastance (H) and scatter plots of H against thoracic gas volume (TGV) with regression lines from analysis of covariance for female (A, B) and male (C, D) vitamin D-replete (white symbols, dashed lines) and -deficient (dark symbols, solid lines) mice at 2 weeks of age.

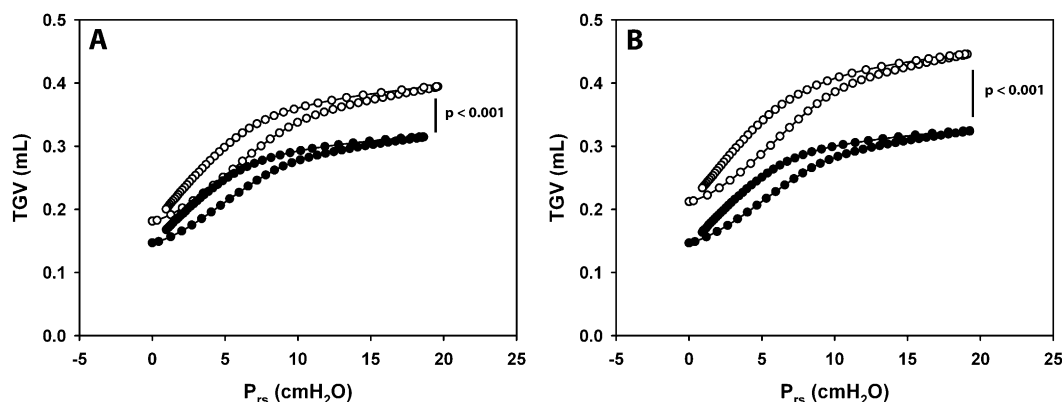


Figure 5. Pressure-volume curves for female (A) and male (B) vitamin D-replete (white symbols) and -deficient (black symbols) mice at 2 weeks of age. Shown are the group mean curves for each group. TGV = thoracic gas volume.

lung volume and mechanics could not be explained by differences in body length in these mice. This observation suggests that vitamin D deficiency has a direct effect on lung growth in the absence of a major effect on somatic growth.

The deficit in lung volume in the vitamin D-deficient mice was substantial (~18% in females and 28% in males). It is important to recognize that this measure of lung volume was made at EELV, which represents the result of opposing forces generated by the elastic recoil of the lung and the outward force generated by the chest wall at zero transrespiratory pressure. Thus, differences in TGV could be influenced by changes in lung structure or differences in the stiffness of the chest wall. This context is important given that vitamin D deficiency alters skeletal muscle growth and function (40). However, it has been shown previously that the chest wall impedance of the mouse is minimal (41), suggesting that the skeletal muscle component of the chest wall is unlikely to have made a significant contribution to the measured TGV in this instance. Additionally, although it is possible that the structural integrity of the ribs may have been altered by vitamin D deficiency, which may influence TGV,

whole-body dual-energy x-ray absorptiometry scans suggested that bone mineral content was not altered by exposure to the vitamin D-deficient diets. This argues against the possibility of altered rib structure contributing to decreases in lung volume and highlights the importance of our calcium supplementation regime, which allowed us to identify the effect of vitamin D deficiency alone. Importantly, this deficit in TGV was maintained over the range of the pressure-volume curve, thus demonstrating a clear effect of vitamin D deficiency, in the absence of hypocalcaemia, on total lung volume.

Differences in lung size, in their own right, may be sufficient to explain altered lung function and respiratory disease prevalence. In humans there is a strong association between low birth weight (as a marker of lung size), lung function later in life (42), and risk of hospitalization caused by respiratory illness (43). This link can be explained intuitively by the smaller airways associated with small lungs resulting in higher resistance to airflow and a decreased capacity to clear pathogens. Not surprisingly, given the large differences in lung volume between the groups of mice, there were substantial differences in lung

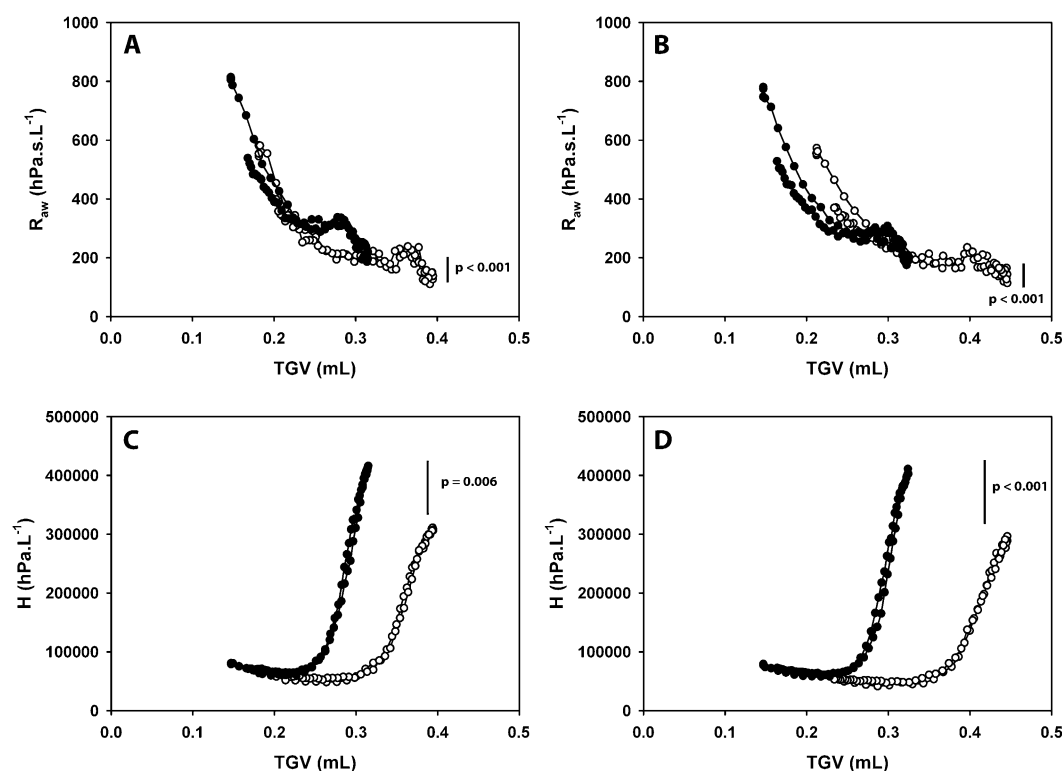


Figure 6. Plots of the volume dependence of airway resistance (R_{aw}) and tissue elastance (H) against thoracic gas volume (TGV) during slow inflation-deflation maneuvers up to 20 cm H_2O transrespiratory pressure in female (A, C) and male (B, D) vitamin D-replete (white symbols) and -deficient (black symbols) mice at 2 weeks of age. Shown are the mean curves for each group.

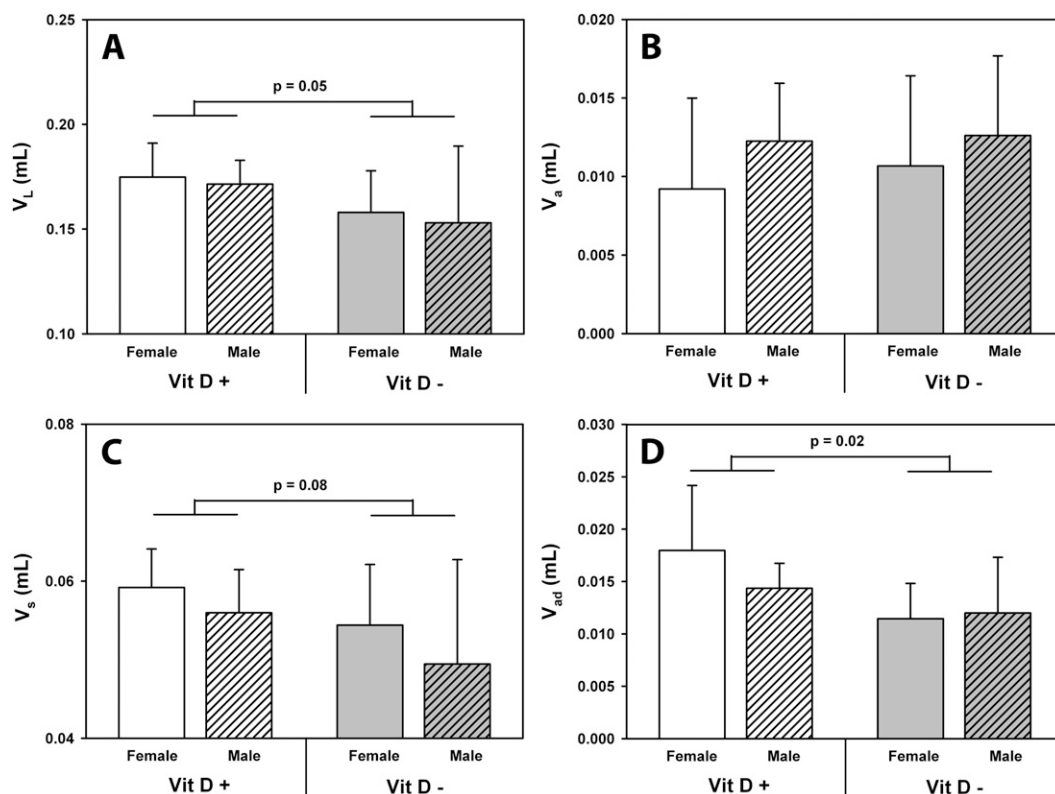


Figure 7. Lung volume (V_L ; A), volume of air in the major airways (V_a ; B), volume of alveolar septa (V_s ; C) and volume of air in the alveolar ducts (V_{ad} ; D) measured by stereology from fixed lungs of 2-week-old female and male (cross hatched) vitamin D-replete (white bars) and -deficient (gray bars) mice. Data are mean (SD).

mechanics. However, these differences in lung volume were not sufficient to explain the differences in lung mechanics we measured. In particular, for a given lung volume, R_{aw} was substantially higher in the deficient mice. G and H could be partially explained by differences in lung volume at EELV; however, this was not the case when the lung was inflated. Specifically, in the anesthetized state we allowed TGV to be self established at EELV, which for the reasons discussed previously may be significantly influenced by chest wall structure. However, by inflating the lungs and tracking lung mechanics we were able to show substantial, physiologically relevant, differences in the volume dependence of lung mechanics in the vitamin D-deficient mice. These differences were particularly evident in the rate of change of parenchymal mechanics with increasing P_{rs} . These data clearly suggest an effect of vitamin D deficiency on the mechanical properties of the lung tissue; however, the nature of this structural difference was not clear from our data.

The primary difference in lung structure between groups, which was consistent between sexes, was a smaller lung volume and size. Differences in the volume of the major airways were not observed. However, we did measure differences in the volume of the alveolar ducts. R_{aw} in the model of the frequency dependence of Z_{rs} that was used in this study represents the frequency-independent Newtonian resistance to flow. In mice, because of the relatively low contribution of the chest wall (41, 44), this primarily reflects resistance of the airways where air moves by bulk flow. The anatomy of the mouse lung, whereby large airways rapidly give way to alveolar ducts (44), is such that the differences we observed between groups in the volume of the alveolar ducts can explain the differences we observed in R_{aw} . At EELV the parenchymal lung mechanics in females was explained entirely by differences in lung volume between groups, whereas this was not the case in male mice. The only structural parameter that showed a different response between males and females was the number of alveoli. The decrease in

the number of alveoli in deficient female mice compared with replete females may explain why the differences in parenchymal mechanics were negligible after adjusting for lung volume. The fact that lung volume did not explain differences in parenchymal mechanics at EELV in male mice suggests a difference in the underlying structure of the lung parenchyma, although the nature of this was not clear from the structural parameters we assessed.

Because of the nature of this study we were not able to determine whether the differences in lung size and function we observed in the offspring were the result of their own deficient status or as a consequence of developmental deficits that

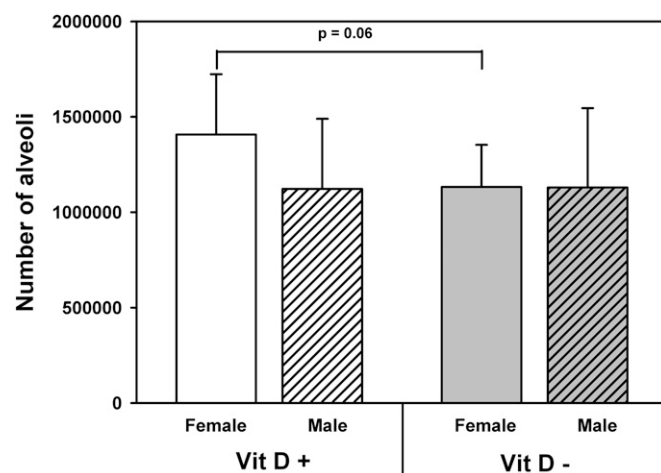


Figure 8. Number of alveoli measured by stereology from fixed lungs of 2-week-old female and male (cross hatched) vitamin D-replete (white bars) and -deficient (gray bars) mice. Data are mean (SD).

occurred *in utero* caused by the mother's deficiency. It is important to note that extreme nutritional manipulation resulting in caloric restriction is a potent model of intrauterine growth restriction. Animal models have been used to demonstrate that *in utero* caloric restriction results in decreased body weight and a lower lung volume to body weight ratio (45). Notwithstanding the issues associated with standardizing lung size by body weight, these data suggest that caloric restriction alters lung growth. In the present study the calorie content of both diets was similar. Additionally, there was little evidence for an effect of exposure to vitamin D-deficient diets on somatic growth suggesting that the mice were not grossly undernourished *in utero*. Thus, the effects of maternal vitamin D status versus the role of the vitamin D status of the individual after birth on lung development in this study could not be distinguished.

For the first time we have demonstrated a direct role for vitamin D in causing decreased lung function in the absence of known confounders, thus confirming the assertion by epidemiologic studies that there is a relationship between vitamin D deficiency and lung function. Specifically, vitamin D deficiency resulted in physiologically significant decreases in lung volume without a major influence on somatic growth. There were corresponding deficits in lung function in the deficient mice that could not be entirely explained by differences in lung volume. There was some evidence to suggest that the structure of the lung was altered because of differences in the volume dependence of lung mechanics at high transrespiratory pressure; however, the nature of this structural difference was not apparent from our data. These differences in lung volume were also apparent histologically. The observed differences in lung volume and lung mechanics, which were substantial and physiologically relevant, raise serious concerns regarding the increased prevalence of vitamin D deficiency in the community and the potential impact this may have on general lung health and in particular susceptibility to obstructive lung disease.

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References

- Nowson CA, Margerison C. Vitamin D intake and vitamin D status of Australians. *Med J Aust* 2002;177:149–152.
- Ginde AA, Liu MC, Camargo CA. Demographic differences and trends of vitamin D insufficiency in the US population, 1988–2004. *Arch Intern Med* 2009;169:626–632.
- Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266–281.
- Dogan M, Erol M, Cesur Y, Yuca SA, Dogan SZ. The effect of 25-Hydroxyvitamin D-3 on the immune system. *J Pediatr Endocrinol Metab* 2009;22:929–935.
- Mathieu C, Gysemans C, Giulietti A, Bouillon R. Vitamin D and diabetes. *Diabetologia* 2005;48:1247–1257.
- Kilkkinen A, Knekt P, Aro A, Rissanen H, Marniemi J, Heliövaara M, Impivaara O, Reunanen A. Vitamin D status and the risk of cardiovascular disease death. *Am J Epidemiol* 2009;170:1032–1039.
- Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? *J Allergy Clin Immunol* 2007;120:1031–1035.
- Litonjua AA. Childhood asthma may be a consequence of vitamin D deficiency. *Curr Opin Allergy Clin Immunol* 2009;9:202–207.
- Janssens W, Lehouck A, Carremans C, Bouillon R, Mathieu C, Decramer M. Vitamin D beyond bones in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2009;179:630–636.
- Janssens W, Bouillon R, Claes B, Carremans C, Lehouck A, Buyschaert I, Coolen J, Mathieu C, Decramer M, Lambrechts D. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. *Thorax* 2010;65:215–220.
- Chishimba L, Thickett DR, Stockley RA, Wood AM. The vitamin D axis in the lung: a key role for vitamin D-binding protein. *Thorax* 2010;65:456–462.
- Camargo CA, Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, Gillman MW. Prospective study of maternal intake of vitamin D during pregnancy and risk of wheezing illnesses in children at age 2 years. *J Allergy Clin Immunol* 2006;117:721–722.
- Camargo CA, Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, Kleinman K, Gillman MW. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. *Am J Clin Nutr* 2007;85:788–795.
- Devereux G, Litonjua AA, Turner SW, Craig LCA, McNeill G, Martindale S, Helms PJ, Seaton A, Weiss ST. Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am J Clin Nutr* 2007;85:853–859.
- Brehm JM, Celedon JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, Laskey D, Sylvia JS, Hollis BW, Weiss ST, *et al*. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med* 2009;179:765–771.
- Searing DA, Zhang Y, Murphy JR, Hawk PJ, Goleva E, Leung DYM. Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use. *J Allergy Clin Immunol* 2010;125:995–1000.
- Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DYM. Vitamin D levels, lung function, and steroid response in adult asthma. *Am J Respir Crit Care Med* 2010;181:699–704.
- Tseng M, Giri V, Bruner DW, Giovannucci E. Prevalence and correlates of vitamin D status in African American men. *BMC Public Health* 2009;9:191.
- Brock K, Huang WY, Fraser DR, Ke L, Tseng M, Stolzenberg-Solomon R, Peters U, Ahn J, Purdue M, Mason RS, *et al*. Low vitamin D status is associated with physical inactivity, obesity and low vitamin D intake in a large US sample of healthy middle-aged men and women. *J Steroid Biochem Mol Biol* 2010;121:462–466.
- Scragg R, Camargo CA. Frequency of leisure-time physical activity and serum 25-hydroxyvitamin D levels in the US population: results from the Third National Health and Nutrition Examination Survey. *Am J Epidemiol* 2008;168:577–586.
- Strine TW, Balluz LS, Ford ES. The associations between smoking, physical inactivity, obesity, and asthma severity in the general US population. *J Asthma* 2007;44:651–658.
- Serres I, Gautier V, Varray A, Prefaut C. Impaired skeletal muscle endurance related to physical inactivity and altered lung function in COPD patients. *Chest* 1998;113:900–905.
- Taher YA, van Esch B, Hofman GA, Henricks PAJ, van Oosterhout AJM. 1 alpha,25-dihydroxyvitamin D-3 potentiates the beneficial effects of allergen immunotherapy in a mouse model of allergic asthma: role for IL-10 and TGF-beta. *J Immunol* 2008;180:5211–5221.
- Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin D and pulmonary function in the Third National Health and Nutrition Examination Survey. *Chest* 2005;128:3792–3798.
- Nguyen M, Trubert CL, Rizk-Rabin M, Rehan VK, Besancon F, Cayre YE, Garabedian M. 1,25-Dihydroxyvitamin D3 and fetal lung maturation: immunogold detection of VDR expression in pneumocytes type II cells and effect on fructose 1,6 biphosphatase. *J Steroid Biochem Mol Biol* 2004;89:93–97.
- Damera G, Fogle HW, Lim P, Goncharova EA, Zhao H, Banerjee A, Tliba O, Krymskaya VP, Panettieri RA. Vitamin D inhibits growth of human airway smooth muscle cells through growth factor-induced phosphorylation of retinoblastoma protein and checkpoint kinase 1. *Br J Pharmacol* 2009;158:1429–1441.
- Brunvand L, Quigstad E, Urdal P, Haug E. Vitamin D deficiency and fetal growth. *Early Hum Dev* 1996;45:27–33.

28. Panda DK, Miao D, Tremblay ML, Sirois J, Farookhi R, Hendy GN, Goltzman D. Targeted ablation of the 25-hydroxyvitamin D 1 alpha-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. *Proc Natl Acad Sci USA* 2001;98:7498–7503.
29. Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, Kawakami T, Arioka K, Sato H, Uchiyama Y, *et al.* Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. *Nat Genet* 1997; 16:391–396.
30. Janosi TZ, Adamicza A, Zosky GR, Asztalos T, Sly PD, Hantos Z. Plethysmographic estimation of thoracic gas volume in apneic mice. *J Appl Physiol* 2006;101:454–459.
31. Zosky GR, Adamicza A, Bozanich EM, Cannizzaro V, Larcombe AN, Turner DJ, Sly PD, Hantos Z. The bimodal quasi-static and dynamic elastance of the murine lung. *J Appl Physiol* 2008;105: 685–692.
32. Fredberg JJ, Stamenovic D. On the imperfect elasticity of lung tissue. *J Appl Physiol* 1989;67:2408–2419.
33. Hsia CCW, Hyde DM, Ochs M, Weibel EW. An official policy statement of the American Thoracic Society/European Respiratory Society: standards for quantitative assessment of lung structure. *Am J Respir Crit Care Med* 2010;181:394–418.
34. Zosky GR, Noble PB, Sly PD. Instillation fixation and overinflation of the mouse lung. *J Appl Physiol* 2010;109:935.
35. Nyengaard JR, Gundersen HJG. Sampling for stereology in lungs. *Eur Respir Rev* 2006;15:107–114.
36. Yan X, Carbayo JJP, Weibel ER, Hsia CCW. Variation of lung volume after fixation when measured by immersion or Cavalieri method. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L242–L245.
37. Fung YC. A model of the lung structure and its validation. *J Appl Physiol* 1988;64:2132–2141.
38. Ochs M. A brief update on lung stereology. *J Microsc (Paris)* 2006;222: 188–200.
39. Cook CD, Helliesen PJ, Agathon S. Relation between mechanics of respiration, lung size and body size from birth to young adulthood. *J Appl Physiol* 1958;13:349–352.
40. Ceglia L. Vitamin D and its role in skeletal muscle. *Curr Opin Clin Nutr Metab Care* 2009;12:628–633.
41. Sly PD, Collins RA, Thamrin C, Turner DJ, Hantos Z. Volume dependence of airway and tissue impedances in mice. *J Appl Physiol* 2003;94:1460–1466.
42. Hancox RJ, Poulton R, Greene JM, McLachlan CR, Pearce MS, Sears MR. Associations between birth weight, early childhood weight gain and adult lung function. *Thorax* 2009;64:228–232.
43. Walter EC, Ehlenbach WJ, Hotchkin DL, Chien JW, Koepsell TD. Low birth weight and respiratory disease in adulthood a population-based case-control study. *Am J Respir Crit Care Med* 2009;180:176–180.
44. Irvin CG, Bates JH. Measuring the lung function in the mouse: the challenge of size. *Respir Res* 2003;4:4.
45. Chen CM, Wang LF, Su BC. Effects of maternal undernutrition during late gestation on the lung surfactant system and morphometry in rats. *Pediatr Res* 2004;56:329–335.

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