The impact of vitamin D on fetal and neonatal lung maturation. A systematic review

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1Hans Christian Andersen’s Children’s Hospital, Odense University Hospital, Odense, Denmark; 2Clinical Institute, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark; 3Institute of Molecular Medicine, Department of Cardiovascular and Renal Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark

Submitted 13 May 2014; accepted in final form 12 January 2015

Lykke Sorensen GL, Beck-Nielsen SS, Christesen HT. The impact of vitamin D on fetal and neonatal lung maturation. A systematic review. Am J Physiol Lung Cell Mol Physiol 308: L587–L602, 2015. First published January 16, 2015; doi:10.1152/ajplung.00117.2014.—Respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD) are major complications to preterm birth. Hypovitaminosis D is prevalent in pregnancy. We systematically reviewed the evidence of the impact of vitamin D on lung development, surfactant synthesis, RDS, and BPD searching PubMed, Embase, and Cochrane databases with the terms vitamin D AND (surfactant OR lung maturation OR lung development OR respiratory distress syndrome OR fetal lung OR prematurity OR bronchopulmonary dysplasia). Three human studies, ten animal studies, two laboratory studies, and one combined animal and laboratory study were included. Human evidence was sparse, allowing no conclusions. BPD was not associated with vitamin D receptor polymorphism in a fully adjusted analysis. Animal and laboratory studies showed substantial positive effects of vitamin D on the alveolar type II cell, fibroblast proliferation, surfactant synthesis, and alveolarization. These data support the hypothesis of hypovitaminosis D as a frequent, modifiable risk factor of RDS and BPD, which should be tested in randomized controlled trials on pregnant women, those with threatening pregnancy, and those with preterm birth. Future experimental and human studies should aim to identify optimal time windows, vitamin D doses, and cut-off levels for 25-hydroxyvitamin D in interventions against RDS, BPD, and late adverse respiratory outcomes.

vitamin D; fetus; lung; surfactant; preterm; neonate

The importance of vitamin D in nonskeletal fetal and childhood health has become increasingly recognized in the last decades (19, 65). The impact of vitamin D on early lung development and maturation and lung diseases of early life is an emerging field of research. Hypovitaminosis D during pregnancy is associated with reduced placental development and weight, leading to a presumed risk of preterm birth (24, 25), which per se may lead to respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD). Moreover, vitamin D is suggested to play a role in the embryogenesis and in cellular growth and differentiation, including lung development and regulation of lung maturation in the fetus (23, 29, 38, 70, 71, 108), whereby hypovitaminosis D could be hypothesized to aggravate premature neonatal lung diseases.

Vitamin D is involved in the development of the innate and adaptive immune system, and, because of its unique capacity to bind to the vitamin D receptor (VDR) and thereby to serve as a transcriptional factor, vitamin D can regulate gene expression and exert widespread immunomodulatory effects including proliferation, differentiation, and complex regulation (19, 56, 58–61, 64, 65, 68, 69, 105). Evidence suggests that vitamin D may stimulate regulatory T cell development and T cell function to suppress inappropriate Th1 and Th2 responses to environmental exposure (i.e., allergens, infection load), leading to a more balanced immune response inhibiting autoimmune diseases (with Th1 dominance) and allergic diseases (Th2 dominance) (55). Vitamin D could therefore be involved in lung diseases of early childhood through immunomodulatory effects.

Although controversial, several epidemiological studies have described an association between maternal vitamin D deficiency and the development of early childhood respiratory infections, wheezing, and asthma later in life (15, 16, 47, 91).

Human lung development begins in the fifth fetal week and continues throughout the first few years of life. It includes five phases: the embryonic, pseudoglandular, canalicular, saccular, and alveolar phase (Fig. 1) (14). The airway tree, pulmonary parenchyma, and vascular connections start to develop during the embryonic and pseudoglandular phases. In the canalicular phase, the peripheral tubules widen, the first air-blood barrier appears, and the epithelium differentiates into type I (ATI) and type II (ATII) alveolar cells. The synthesis of surfactant begins...
at the end of this phase during the 24th week and increases as the pulmonary parenchyma grow during the first half of the saccular phase with completion by week 34. In the alveolar phase, more alveoli are formed, providing an increase in the gas-exchange surface area, beginning week 38 and continuing postpartum, especially during the first 1–3 yr of life, with completion when the child is ~8 yr old (14).

Contrary to humans, the expression of surfactant in rats starts in the saccular phase on the 19th gestational day and is completed on the 21st-22nd day (at term) (86). The alveolar phase in rats does not begin until postpartum at postnatal days 4–5 and slowly progresses throughout life (7, 66, 72) (Fig. 1). Accordingly, rats are born at a lung developmental stage equivalent to that of a preterm human neonate (72), making rats highly suitable as an animal model for human lung diseases of the preterm.

Pulmonary surfactant is a lipoprotein complex located on the surface of the lung alveoli. Surfactant reduces surface tension and participates in host defense and the control of inflammation in the lung (75). Surfactant is synthesized and secreted by the ATII pneumocytes. In addition, the ATII cells are responsible for the regulation of alveolar ion transport. The cells are characterized by specific organelles, the osmiophilic lamellar bodies (OLBs), which represent the intracellular storage site of lung surfactant. The ATII cells are in direct contact with the ATI cells above the basement membrane and fibroblasts and other interstitial cells beneath the membrane. Fibroblasts are recognized as influencing surfactant biosynthesis through paracrine actions. Before parturition, fibroblasts secrete a polypeptide that stimulates the rate-limiting enzyme for surfactant synthesis by the ATII cells (34, 60, 70). Perinatal lung maturation is a highly regulated process, and alveolar epithelial-mesenchymal interactions play a critical role (90, 96).

Surfactant consists of 90% phospholipids and 10% proteins. The major phospholipid component dipalmitoylphosphatidylcholin reduces the surface tension in the alveoli and maintains alveolar expansion at the end of expiration. The proteins are mainly the four apoproteins, surfactant protein A, B, C, and D (SP-A, SP-B, SP-C, and SP-D). The hydrophilic SP-A and SP-D apoproteins are a defense against infections and inflamm-

**Fig. 1. Phases of lung maturation and synthesis of surfactant in humans and rats.**
monary surfactant synthesis (22a). The most dramatic improvement in the treatment of RDS was introduced in the early 1990s with the possibility of intratracheal surfactant therapy, leading to decreased mortality and morbidity (36). Despite improved treatment, RDS continues to be a severe, high-mortality disease in the extremely premature. In survivors, RDS may be complicated by BPD, a “chronic” lung disease characterized by impaired alveolar development and inflammation (35). The current RDS therapy with surfactant is expensive, and, as exogenous surfactant does not increase the local surfactant production, repeated administration may be needed (92).

Vitamin D₃, also known as cholecalciferol, is a fat-soluble secosteroid and is either synthesized from 7-dehydrocholesterol in the skin after ultraviolet B (sunlight) exposure or obtained from food of animal origin. Vitamin D₂ or ergocalciferol is obtained only from food of plant origin. Both forms of vitamin D may also be obtained by vitamin D supplementation. Vitamin D is converted in the liver to 25(OH)D, and the further classical pathway involves renal conversion of 25(OH)D to the biologically active form 1,25(OH)₂D, also known as calcitriol (38, 45). A more recently described pathway involves nonrenal uptake of 25(OH)D, either free or bound to vitamin D-binding protein (VDBP, also known as Gc-globulin), followed by intracellular 1α-hydroxylation to 1,25(OH)₂D in almost all tissues (38, 45, 80). Metabolites in the vitamin D pathway are transported in the circulation predominantly bound to VDBP and albumin, with <1% in the free, bioavailable form (17, 38). VDBP regulates availability in tissues, allowing only the small free fraction of vitamin D metabolites to passively enter cells through diffusion across cell membranes (17).

Moreover, VDBP has immunomodulatory functions primarily related to macrophage activation and neutrophil chemotaxis, and variations in the gene are associated with airway diseases, such as asthma and chronic obstructive pulmonary disease (17). The human VDBP gene is highly polymorphic, and the ability to bind vitamin D and respond to vitamin D supplementation varies according to genotype. Largely because of two frequent VDBP polymorphisms, black Americans had lower VDBP concentrations compared with whites but similar estimated bioavailable 25(OH)D despite lower total serum 25(OH)D concentrations (77). As a consequence, the widely accepted serum total 25(OH)D cut-off values of 25, 50, and 75 nmol/l for vitamin D deficiency, insufficiency, and optimal status, respectively (3, 45, 46, 83, 98), should be used with caution across racial genetic differences. Moreover, use of elder routine 25(OH)D assays may differ 20% below or above the modern liquid chromatography-tandem mass spectrometry golden-standard method (26). Given these limitations, the vitamin D supplementation dose is a superior measure compared with achieved 25(OH)D concentrations in evaluation of vitamin D status, especially across races (12).

In white Europeans, genome-wide association studies have shown that variants near genes involved in cholesterol synthesis (the 7-dehydrocholesterol reductase gene DHCR7/NA-DSYNS), 25-hydroxylation (CYP2R1), vitamin D transport (the VDBP gene GC), and degradation (the 24-hydroxylase gene CYP24A1) affect the S-25(OH)D concentration (101). The actions of 1,25(OH)₂D₃ are mediated through binding to the nuclear VDR, a member of the superfamily of steroid receptors (45, 68, 69). VDR is a ligand-dependent transcription factor that alters the expression of select target genes in response to 1,25(OH)₂D₃ (38, 76). The VDR-ligand complex forms a heterodimer with the retinoid-X receptor (RXR) and binds vitamin D-responsive elements (VDREs) located predominantly in the promoter regions of target genes (53, 76). Likewise, the vitamin A-derivate retinoic acid (RA) binds to the retinoic acid receptor (RAR). The 1,25(OH)₂D₃-VDR complex and RA-RAR complex can form heterodimers and thereby transduce the hormonal signal into altered gene transcriptional events. In combination with transcription factors and coregulatory proteins, the 1,25(OH)₂D₃-VDR complex promotes or suppresses the transcription of a wide range of genes (76). VDR knockout in mice results in early-onset emphysema and decline in lung function, with lymphoid aggregate formation secondary to inflammatory changes (94). More subtle mutations or polymorphisms in the VDR gene, which may affect gene promotion, RNA transcription efficacy, or protein structure, have been associated with a variety of diseases, such as multiple sclerosis, type I diabetes mellitus, osteoporosis, and colon, breast, and prostate cancer (49, 74, 97). The impact of VDR knockout on the fetal and early postnatal lung has, to our knowledge, not been studied.

Early lung development studies on the effect of DNA variations in the vitamin D synthesis, transport, degradation, and action from polymorphisms to gene knockout are generally still missing. Taken together, data on common vitamin D-related polymorphisms should optimally be included in studies reporting on vitamin D doses or serum 25(OH)D levels.

During pregnancy, the maternal requirements of vitamin D are increased because of several modifications of the vitamin D metabolism. Maternal plasma levels of 25(OH)D do not change significantly during normal pregnancy if the intake of vitamin D and sun exposure remain unchanged. In contrast, circulating levels of active 1,25(OH)₂D₃ increase several times over from early pregnancy and remain high during the entire pregnancy (32, 45, 73). The fetus has no endogenous production of 25(OH)D and is fully dependent on the transfer from the mother. Whereas 25(OH)D passes the placenta, 1,25(OH)₂D₃ is restricted to the maternal circulation and is believed to be produced de novo in the placenta and in the fetus itself (23, 32, 33, 45, 104). During pregnancy and in early childhood, the interpretation of serum 25(OH)D levels may be complicated by the presence of the inactive or less biologically active C3-epimer of 25(OH)D (6, 106). It is suggested that the C3-epimer is generated within the fetal-placental unit and not transferred across the placenta as efficiently as 25(OH)D₃ (6). Hypovitaminosis D is frequent in pregnant women and in neonates (3, 22, 39, 45, 46, 84, 98), and, because the transplacental transfer of 25(OH)D mainly occurs during the last trimester, preterm infants are particularly at risk of vitamin D deficiency (65).

Taking the knowledge of a potential role of vitamin D in lung development and maturation into account, we raised the hypothesis that hypovitaminosis D could be a frequent yet largely unrecognized and modifiable risk factor of RDS and BPD in premature neonates. We therefore aimed to systematically review the evidence on the impact of vitamin D on lung development and maturation, including synthesis of surfactant, and the impact on the development of RDS and BPD.

AJP-Lung Cell Mol Physiol • doi:10.1152/ajplung.00117.2014 • www.ajplung.org
Methods

Our systematic review was based on the guidelines of Transparent Reporting of Systematic Reviews and Meta-analysis (PRISMA statement 2009) (52).

Search strategy. For the systematic review, the databases PubMed and Embase were searched using the term vitamin D AND (surfactant OR lung maturation OR lung development OR respiratory distress syndrome OR fetal lung OR prematurity OR bronchopulmonary dysplasia). No additional titles were found using the MeSH terms or by search in the Cochrane Database. Additional studies were identified by manual search of reference lists of all full-text publications selected. Human, animal, and laboratory studies in English written language were included without time limit. The last search was made on January 12, 2015.

Exclusion criteria were as follows: 1) non-English language, 2) reviews or commentaries, and 3) topics not related to the review (studies without data on vitamin D in newborns, fetal and/or neonatal lung maturation or development, surfactant production, or respiratory problems attributable to altered lung function in newborns).

Broader nonsystematic searches were performed to serve as background material, including vitamin D AND (prematurity OR fetal immune system OR immune system development OR glucocorticoid interaction OR retinoic acid interaction).

Screening and selection. After duplicates were removed, a screening of title and abstract was undertaken by two authors (S. Lykkedegn and H. Christesen). The remaining studies of possible relevance were obtained in full text, and the reviewers made an independent assessment. Disagreements between the reviewers were resolved by discussion until consensus was achieved.

Risk of bias. To access the risk of bias of RCTs, the Cochrane Collaboration has developed the Cochrane RoB Tool (41). Systematic reviews of experimental animal studies are not yet common practice, and just recently the SYstematic Review Centre for Laboratory animal Experimentation has presented a SYRCLE’s RoB Tool for animal studies (42). The SYRCLE’s RoB Tool is based on the Cochrane RoB Tool and has been adjusted for the aspects of bias known to play a role in animal intervention studies. We assessed the risk of bias in the included RCTs and animal intervention studies using the above-mentioned tools (Table 1).

Results

Our search identified 1,324 records, of which 246 were duplicates (Fig. 2). After application of the exclusion criteria, 16 publications remained. The study characteristics are summarized in Tables 2,3, and 4. Only three human studies were included without time limit. The last search was made on January 12, 2015.

In 1999, Backström et al. (5) randomized 39 premature children born before the 33rd gestational week to vitamin D 200 IU/kg per day (maximum 400 IU/day) or 960 IU/day until 3 mo of age. Gestational age, birth weight, and birth length were comparable between the groups. At birth, the mean serum 25(OH)D concentrations were 29.8 vs. 29.2 nmol/l. At 6 wk of age, 25(OH)D was significantly higher in the high-dose vitamin D group (mean 66.7 vs. 45.7 nmol/l). The authors focused on infant bone mineralization obtained by dual-energy X-ray absorptiometry scans but noted, as the only significant finding, a reduced need for assisted ventilation in the high-dose vitamin D group (median 0 vs. 4 days, P = 0.01). Moreover, a trend toward a lower duration of oxygen supplementation was recorded (median 2 vs. 14 days, P = 0.06). Respiratory acidosis was more prevalent in the low-dose vitamin D group.

In 2013, Ataseven et al. (4) performed an observational cohort study investigating vitamin D deficiency as a risk factor for RDS. A total of 152 infants with a gestational age of 29 to 35 wk was included. Data on sex, gestational age, body weight, Apgar scores, antenatal steroid use, type of delivery, and antenatal problems were recorded, and serum 25(OH)D and calcium levels were measured. All 152 infants had 25(OH)D below 75 nmol/l (64% below 25 nmol/l, 33% 25–50 nmol/l, and 3% 50–75 nmol/l). No correlation between gestational age and vitamin D status was found. In unadjusted analysis, RDS was seen in 28% of the group with 25(OH)D < 25 nmol/l compared with 14% in those with higher 25(OH)D. RDS was reduced 3.34 times in newborns with higher vitamin D levels, but no multivariate analysis was done.

In an observational study from 2014, Koroglu et al. (50) found an increased adjusted odds ratio (OR = 4.11, 95% CI 1.08–15.68; P = 0.038) for BPD among 109 preterm babies with the variant VDR Fok 1 polymorphism Ff or ff, independent of gestational age, or birth weight, they saw no effect of variant Fok 1.

Animal studies. The ten animal studies (23, 59 – 61, 68 – 71) all showed a positive impact of vitamin D on fetal and neonatal lung development and maturation. These effects included a VDR-dependent increase in the synthesis and secretion of surfactant phospholipids in ATII cells and a postnatal vitamin D-dependent alveolar growth. The presence of the VDR in the fetal lung was restricted to the late period of gestation, corresponding to the onset of the surfactant synthesis and to the differentiation of the ATII cells.

In 1987, Nguyen et al. (68) identified a significant amount of VDR related to pneumocytes in the fetal rat lung during the last quarter of gestation (days 19 – 21 of gestation). Crude and partially purified lung cytosols were incubated in the absence or presence of either 1.500 nM unlabeled 1,25(OH)2D3 in increasing concentrations or a 50-fold excess of radioinert 1,25(OH)2D3. The amount of VDR was either calculated by Scatchard analysis or a result of measured radioactivity using a liquid scintillation spectrometer. This study suggested that pneumocytes might be a major target tissue for 1,25(OH)2D3 during fetal life.

In a subsequent study from 1990 (69), the group investigated which cell types were directly responsive to 1,25(OH)2D3 during fetal life and whether the physiological downregulation of the VDR that occurs in the adult lung also was detectable during the perinatal period.

In vivo, lung tissue from rat fetuses (day 21) and newborn rats (3 days after birth) were prepared for immunohistochemical staining with a monoclonal antibody against VDR (9A7γ). Furthermore, the level of VDRs was estimated in cytosols from
fresh lung tissue using a 50-fold excess of unlabeled 1,25(OH)2D3, as previously described by the group (68). The number of specific VDRs was calculated as the difference between total binding and nonspecific binding. The highest levels of VDR were located in cells corresponding to ATII cells at the end of pregnancy (days 20–21), whereas little or no immunostaining was observed in the tissue from the 3-day-old pups. The level of VDRs decreased a few hours before delivery and remained low during the first 5 days of life.

In vitro, the level of VDRs was estimated in cytosols from fetal lung explants taken on days 20–21 of gestation and cultured for 48 h using the same method as in vivo. The number of VDRs in vitro was different from the in vivo pattern, as the number did not decrease during the culture period (48 h). In addition, the group used the fetal lung explants to test the effect of factors known to affect lung maturation, including 1,25(OH)2D3, dexamethasone, thyroxine, prolactin, terbutaline, retinoic acid, insulin, and oxytocin.

Table 1. Risk of bias

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<tbody>
<tr>
<td>Random sequence generation (selection bias)</td>
<td>High risk of bias</td>
<td>No description of the randomization of animals</td>
<td>Low bias of risk</td>
<td>Female BALB/c mice were assigned to 2 different diets before mating</td>
<td>Low risk of bias</td>
<td>Pregnant rats were randomly assigned to 3 different groups</td>
<td>Low risk of bias</td>
<td>The preterm infants were randomly assigned to 2 groups within 48 h of birth</td>
</tr>
<tr>
<td>Baseline characteristics* (selection bias)</td>
<td>Unclear risk of bias</td>
<td>The relevant baseline characteristics* were not well described or adjusted for in the analysis</td>
<td>Low risk of bias</td>
<td>The relevant baseline characteristics* were well described and adjusted for in the analysis</td>
<td>Unclear risk of bias</td>
<td>The allocation was based on serum levels of vitamin D</td>
<td>Low risk of bias</td>
<td>The allocation was based on bone densitometry and determination of serum vitamin D metabolites</td>
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<td>Low risk of bias</td>
<td>The allocation was based on serum levels of vitamin D</td>
<td>Low risk of bias</td>
<td>The allocation was based on serum levels of vitamin D</td>
<td>Low risk of bias</td>
<td>The allocation was based on serum levels of vitamin D</td>
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<td>Random housing (performance bias)</td>
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<td>Unclear risk of bias</td>
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<td>Unclear risk of bias</td>
<td>No information on how the newborn rats were housed with the foster mother</td>
<td>High risk of bias</td>
<td>No information on how the newborn rats were housed with the foster mother</td>
</tr>
<tr>
<td>Blinding (performance bias)</td>
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<td>No information on blinding of the caretakers</td>
<td>High risk of bias</td>
<td>No information on blinding of the caretakers</td>
<td>High risk of bias</td>
<td>Injection were given counterclockwise from right ovary</td>
<td>High risk of bias</td>
<td>No information on how the newborn rats were housed with the foster mother</td>
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<tr>
<td>Random outcome assessment (detection bias)</td>
<td>Unclear risk of bias</td>
<td>No description of how the animals were selected</td>
<td>Unclear risk of bias</td>
<td>No description of how the animals were selected</td>
<td>Low risk of bias</td>
<td>Injection were given counterclockwise from right ovary</td>
<td>Low risk of bias</td>
<td>No information on how the newborn rats were housed with the foster mother</td>
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<tr>
<td>Incomplete outcome data (attrition bias)</td>
<td>High risk of bias</td>
<td>No description of exclusion criteria or group sizes</td>
<td>Low risk of bias</td>
<td>No exclusion criteria were described, groups are equal in size</td>
<td>Low risk of bias</td>
<td>The study protocol was not available in the manuscript, but the report includes all expected outcomes</td>
<td>Low risk of bias</td>
<td>All prespecified outcomes were reported</td>
</tr>
<tr>
<td>Selective outcome reporting (reporting bias)</td>
<td>Unclear risk of bias</td>
<td>The study protocol was not described in the manuscript, all expected outcomes are described</td>
<td>Low risk of bias</td>
<td>The study protocol was described in the manuscript, all expected outcomes are described</td>
<td>Low risk of bias</td>
<td>The study protocol was not available in the manuscript, but the report includes all expected outcomes</td>
<td>Low risk of bias</td>
<td>The study protocol was not available in the manuscript, but the report includes all expected outcomes</td>
</tr>
<tr>
<td>Other bias</td>
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<td>No other problems that could result in high risk of bias</td>
<td>Low risk of bias</td>
<td>No other problems that could result in high risk of bias</td>
<td>Unclear risk of bias</td>
<td>The form and dose of vitamin D were not described</td>
<td>Unclear risk of bias</td>
<td>No information on how they increase the dose of vitamin D in group A, no description on the type of assisted ventilation</td>
</tr>
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</table>

*Bias characteristics: feed of the mother (content of vitamin D, calcium, and phosphorus), litter size, sex, age, saturation, hypothermia, body weight, and housing conditions.
Prolactin, thyroxine, 1,25(OH)₂D₃, and to a lesser extent dexamethasone increased the capacity of ATII cells to bind 1,25(OH)₂D₃ by inducing an increase in the number of VDRs without altering the receptor-binding affinity.

The same year Marin et al. (61) demonstrated that 1,25(OH)₂D₃ (10⁻⁹ M) treatment of 18-day-old immature fetal rat lung significantly increased the levels of surfactant-related phospholipids ex vivo. Lipids were extracted from fetal lung explants, and the levels of phospholipids were visualized by thin-layer chromatography. An increase in the surfactant-related phospholipids was also seen in dexamethasone-treated explants, but not to the same extent. Dexamethasone (10⁻⁷ M) did not have any additive effect when present together with 1,25(OH)₂D₃ in the culture medium. Morphological observations were studied by light and electron microscopy. Whereas surfactant mainly was accumulated intracellularly in the dexamethasone-treated explants causing abnormally large OLBs, it was mainly found extracellularly in the luminal space in the 1,25(OH)₂D₃-treated explants. Thus 1,25(OH)₂D₃, not only stimulated the synthesis of phospholipids, but also triggered the surfactant secretion.

The same group used a similar setup in 1993 to analyze the morphological changes in surfactant synthesis and release induced by 1,25(OH)₂D₃ (10⁻⁹ M) and dexamethasone (10⁻⁷ M) (60). Changes were visualized by both light and electron microscopy. They showed no significant effect of 1,25(OH)₂D₃ on the number of immature pneumocytes entering differentiation and no significant effect on structural changes (increase of

Fig. 2. Study flow diagram.

Table 2. Study characteristics, human studies

<table>
<thead>
<tr>
<th>Source</th>
<th>Population</th>
<th>Study Design</th>
<th>Intervention</th>
<th>Outcome</th>
<th>Results</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Backström et al. 1999 (5)</td>
<td>39 preterm infants RCT 200 IU/kg body wt per day ≤400 IU/day (a) vs. 960 IU/day (b)</td>
<td>Gestational age 30 ± 3 (a) vs. 30 + 6 (b) Birth weight 1,365 g (a) vs. 1,510 g (b) Birth length 39 cm (a) vs. 40 cm (b) Duration of assisted ventilation, days 4 (a) vs. 0 (b) Duration of oxygen supplement, days 14 (a) vs. 2 (b)</td>
<td>P = 0.19 P = 0.45 P = 0.65 P = 0.01 P = 0.06</td>
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<tr>
<td>Ataseven et al. 2013 (4)</td>
<td>152 preterm infants Observational cohort study</td>
<td>Gestational age 18 vs. 63 Birth weight, g 1,667 ± 505 vs. 1,974 ± 585 Antenatal corticosteroids, % 23 vs. 32 25(OH)D₃, ng/ml 7.5 ± 4.9 vs. 9.6 ± 5.7</td>
<td>P = 0.01 P = 0.00 P = 0.40</td>
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<tr>
<td>Koroglu et al. 2014 (50)</td>
<td>109 preterm infants Observational cohort study</td>
<td>Gestational age 30.17 vs. 27.19 Birth weight, g 1,523.79 vs. 980.04 Surfactant treatment, % 29 vs. 83 25(OH)D₃, ng/ml 3.59 vs. 41.95 Duration of assisted ventilation, days 3.37 vs. 79.02 Duration of oxygen therapy, days 62 vs. 43</td>
<td>P &lt; 0.001 P &lt; 0.001 P &lt; 0.001 P &lt; 0.001</td>
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RCT, randomized controlled trial. (a), Group receiveing 200 IU/kg body wt per day ≤400 IU/day intervention; (b), group receiving 900 IU/day intervention.
Table 3. Study characteristics, animal studies

<table>
<thead>
<tr>
<th>Source</th>
<th>Animals</th>
<th>Endpoints</th>
<th>Analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nguyen et al.</td>
<td>Sprague-Dawley rats</td>
<td>Distribution of VDR during the last quarter of pregnancy</td>
<td>Binding studies</td>
<td>Significant amounts of VDR were identified in the fetal rat lung during the last quarter of gestation.</td>
</tr>
<tr>
<td>1987 (68)</td>
<td></td>
<td>Light microscopy</td>
<td>Immunohistochemistry</td>
<td>In vivo, the number of VDRs decreased a few hours before delivery and remained low during the first 5 days of life. The highest levels of VDR were located to ATII cells at the end of gestation.</td>
</tr>
<tr>
<td>Nguyen et al.</td>
<td>Sprague-Dawley rats</td>
<td>Localization of VDR</td>
<td>Binding study</td>
<td>In vitro, the number of VDR did not decrease during the culture period. Prolactin, thyroxine, 1,25(OH)2D3 and to lesser extent dexamethasone increased the capacity of ATII cells to bind 1,25(OH)2D3.</td>
</tr>
<tr>
<td>1990 (69)</td>
<td></td>
<td>Light microscopy</td>
<td></td>
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<tr>
<td>Marin et al.</td>
<td>Sprague-Dawley rats</td>
<td>Content of surfactant-related phospholipid content</td>
<td>Thin-layer chromatography (a)</td>
<td>1,25(OH)2D3 significantly increased the levels of phospholipids. Dexamethasone also increased the levels of phospholipids but not to the same extent, and no additive effects of the 2 treatments were shown.</td>
</tr>
<tr>
<td>1990 (61)</td>
<td></td>
<td>Morphology (a)</td>
<td>Electron microscopy (b)</td>
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<tr>
<td>Marin et al.</td>
<td>Pregnant Sprague-Dawley</td>
<td>Morphology</td>
<td>Electron microscopy Light microscopy</td>
<td>1,25(OH)2D3 had no significant effect on either the number of immature pneumocytes entering differentiation or the structural changes in the ATII cells but stimulated the release of surfactant. Dexamethasone slowed down the enlargement of the luminal space significantly.</td>
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<tr>
<td>1993 (60)</td>
<td>rats</td>
<td>Light microscopy Light microscopy (b)</td>
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<td>Edelson et al.</td>
<td>Pathogen-free Wistar rats</td>
<td>Influence of 1,25(OH)2D3 on alveolar epithelial proliferation</td>
<td>Thymidine incorporation Cell number</td>
<td>1,25(OH)2D3 increased thymidine incorporation into DNA in the late neonatal and adult ATII cells but not in fetal or early neonatal ATII cells. The increase in the thymidine incorporation was accomplished by an increase in cell number.</td>
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<td>1993 (23)</td>
<td>Male adult and neonatal</td>
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<td>Autoradiography Flow cytometry</td>
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<td>Sprague-Dawley</td>
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<td>Nguyen et al.</td>
<td>Sprague-Dawley rats</td>
<td>Localization of VDR (a)</td>
<td>Immunohistochemistry (a)</td>
<td>Whereas HPLC was used to demonstrate a paracrine system during the last 3 days of pregnancy (days 19–21 of gestation), immunostaining showed that ATII express VDR and fibroblasts do not. Thin-layer chromatography showed that 1,25(OH)2D3 stimulated the production and release of phospholipids by ATII cells.</td>
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<td>Nguyen et al.</td>
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<td>Phospholipid synthesis and release (d)</td>
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<td>Metabolism of 1,25(OH)2D3 (e)</td>
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<td>Sprague-Dawley rats</td>
<td>VDR labeling</td>
<td>Electron microscopy</td>
<td>VDRs were located to the ATII cell nucleus, cytoplasm, and endoplasmic reticulum at 21 days of gestation. 1,25(OH)2D3 was especially active in the intermediate stage of ATII cell differentiation.</td>
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<td>2009 (85)</td>
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<td>Zosky et al.</td>
<td>Newborn BALB/c mice</td>
<td>Lung volume (TGV) (a)</td>
<td>Plethysmography (a)</td>
<td>In vitro, incubation with 1,25(OH)2D3 or 1,25(OH)2D3-3-epi-D3 and key markers for alveolar epithelial-mesenchymal interactions caused increases in PThrP receptor, PPAR-γ, and ADRP in lipofibroblasts and in SP-B and leptin receptor in ATII cells. Furthermore, both treatments caused a dose-dependent increase in the proliferation of both lipofibroblasts and ATII cells by inhibiting apoptosis.</td>
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<td>2011 (108)</td>
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<td>A modified low-frequency forced-oscillation technique (b)</td>
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<td>Effects of vitamin D on isolated ATII cells and the survival of neonatal rat pups</td>
<td>Survival rate, Oxygenation, Immunohistochemistry, ATII cell growth assay</td>
<td>Antenatal vitamin D treatment improved oxygenation (78% vs. 87%) and survival (64% vs. 57%) significantly after endotoxin exposure when compared with endotoxin alone. Early vitamin D treatment improved alveolar growth by 45% through the first 14 days of life.</td>
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<td>The total pulmonary compliance was not significantly different in the 4 groups, but the tracheal contractility response to acetylcholine showed highest contractility in vitamin D-deficient and 1,000 IU/kg cholecalciferol-supplemented animals compared with 500 IU/kg cholecalciferol-supplemented animals. Serum 25(OH)D was lowest in the non-supplemented group, and levels increased with increased dose of cholecalciferol in the diet. Serum levels of calcium were unaffected by both vitamin D deficiency and supplemented cholecalciferol levels. Similarly, the levels of alkaline phosphatase were unaffected except in the 1,000 IU/kg cholecalciferol-supplemented group, where a significant decrease was seen. Perinatal supplementation with 500 IU/kg cholecalciferol appeared to block the altered airway contractility and alveolar epithelial-mesenchymal signaling caused by vitamin D deficiency.</td>
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VDR, vitamin D receptor; ATII, alveolar type II cell; HPLC, high-performance liquid chromatography; PTHrP, parathyroid hormone-related protein; PPAR-γ, peroxisome proliferator-activated receptor-γ; ADRP, adipocyte differentiation-related protein; SP-B, surfactant protein B; TGV, thoracic gas volume. Letters in parentheses connect the endpoints in the analysis.

the surface area of the epithelial structures and the numbers of cells in the ATII cells. In contrast, the presence of dexamethasone slowed down the enlargement of the luminal space significantly. 1,25(OH)2D3 stimulated the release of surfactant by facilitating the secretion of OLBs into the lumina, whereas dexamethasone significantly inhibited OLB exocytosis, causing an accumulation within the ATII cells.

Later the same year, Edelson et al. (23) examined the effect of 1,25(OH)2D3 on alveolar epithelial proliferation in distal epithelial cells from fetal (gestational days 18, 19, and 22), neonatal (7 and 18 days postpartum), and adult rat lung. Primary cultures of ATII cells were exposed to 1,25(OH)2D3 (10−9 M) and [3H]thymidine, and autoradiographs were made. They showed that 1,25(OH)2D3 acts to increase thymidine incorporation into DNA in primary cultures of late neonatal and adult rat ATII cells but not in fetal or early neonatal ATII cells. The increase in the thymidine incorporation was accompanied by an increase in cell number, demonstrating that 1,25(OH)2D3 acts as a growth factor for ATII cells in postnatal animals.

In 1996, Nguyen et al. (70) showed that ATII cells, but not fibroblasts, express VDR. They demonstrated the presence of a paracrine system, which was activated during the last 3 days of pregnancy (days 19–21 of gestation). Lung fibroblasts and ATII cells isolated from 21-day-old rat fetuses were cultured in the presence of 1,25(OH)2D3 (10−9 M), and a monoclonal antibody against VDR (9A7γ) was used to visualize the receptor by immunostaining. The receptor expression was increased when the isolated ATII cells were incubated with 1,25(OH)2D3 for at least 24 h in culture, indicating that fetal ATII cells express a functional VDR that can be upregulated by 1,25(OH)2D3. To study the ability of fibroblasts and ATII cells to convert 25(OH)D3 into 1,25(OH)2D3, the cells were incubated with [3H]25(OH)D3, and unlabeled synthetic 1,25(OH)2D3 (100 ng) was added to the extracts before the samples were chromatographed by high-performance liquid chromatography (HPLC). Radioactivity was measured by liquid-scintillation spectroscopy and used to calculate the rate of conversation of 25(OH)D3 to 1,25(OH)2D3. The results showed that lung fibroblasts, but not ATII cells, were capable of converting 25(OH)D3 into 1,25(OH)2D3 during the last days of gestation. For the purpose of studying the synthesis of phospholipids, ATII cells were isolated from 20-day-old rat fetuses and cultured in the presence of 1,25(OH)2D3 (10−9 M) or EB-1213 (10−9 M) followed by incubation with [methyl-3H]choline. Phospholipids were extracted, and the incorporated radioactivity was measured. To study the release of phospholipids, the cultured ATII cells were incubated with 1,25(OH)2D3 (10−9 M) or EB-1213 (10−9 M) and prepared for thin-layer chromatography. 1,25(OH)2D3 stimulated the synthesis and secretion of phospholipids by ATII cells significantly compared with controls. The authors suggested on that basis that 1,25(OH)2D3 might be useful in the prevention or treatment of RDS.

Eight years later in 2004, Nguyen et al. (71) discovered that VDRs were located in the nucleus, cytoplasm, and endoplasmic reticulum of ATII cells at 21 days of gestation in fetal rat lungs. Lung tissue from rat fetuses (day 21) was fixed in formaldehyde and incubated with an antibody against VDR (9A7γ). The receptor was visualized by electron microscopy. Furthermore, an association study between VDR expression and ATII cell maturation indicated that 1,25(OH)2D3 was especially active in the intermediate stage of ATII cell differentiation, when the decrease in glycolgen content begins. The study brought further evidence for a physiological role of 1,25(OH)2D3 during the maturation of ATII pneumocytes.
In 2009, Sakurai et al. (85) determined a role of both 1,25(OH)2D3 and its metabolite 1,25(OH)2D3-3-epi-D3 in key alveolar epithelial-mesenchymal interactions.

Embryonic day 19 primary rat lung lipofibroblasts and ATII cells were incubated with 1,25(OH)2D3 (10−11 M) or 1,25(OH)2D3-3-epi-D3 (10−8 M) in vitro, and key markers for alveolar epithelial-mesenchymal interactions were visualized by Western blotting. Both treatments caused increases in the expression of those markers. Whereas treatment of lipofibroblasts caused increases in parathyroid hormone-related protein receptor, peroxisome proliferator-activated receptor-γ (PPAR-γ), and adipocyte differentiation-related protein (ADRP), treatment of ATII cells resulted in an increase in both SP-B and leptin receptor. Furthermore, both 1,25(OH)2D3 and 1,25(OH)2D3-3-epi-D3 treatments caused a dose-dependent increase in the proliferation of both lipofibroblasts and ATII cells by inhibiting apoptosis.

Newborn rat pups were administrated with 1,25(OH)2D3 (10 ng/kg body wt) or 1,25(OH)2D3-3-epi-D3 (50 ng/kg body wt) intraperitoneally during the first 2 wk of postnatal life, and the effects on markers of spontaneous lung maturation were determined by Western blotting. Compared with controls, both treatments led to an increase in the expression of key markers for both lipofibroblast (PPAR-γ and ADRP) and ATII cell (SP-B and SP-C) differentiation. In addition, immunohistochemical staining of lungs from 1,25(OH)2D3-treated animals showed a significant increase in both alveolar count and alveolar septal thickness compared with controls. Altogether, this study implied an essential physiological role of vitamin D in perinatal pulmonary maturation.

In 2011, Zosky et al. (108) showed a connection between vitamin D deficiency and altered postnatal lung development. Female BALB/c mice were assigned to either a vitamin D-deficient or vitamin D-replete (2.195 IU/kg) diet. Pups of both sexes were studied at 2 wk of age. Whereas lung volume (thoracic gas volume [TGV]) and lung mechanics were assessed through respectively plethysmographic measurements and modified low-frequency forced-oscillation technique, respectively, lung structure was assessed after fixation with 2.5% glutaraldehyde and visualized by stereology. The results showed significantly smaller TGV and significantly higher airway resistance and lung mechanics (tissue damping and elastance) in vitamin D-deficient mice compared with replete controls, which still after adjustment for body length suggest that vitamin D has an effect on postnatal lung growth. Stereology also showed significantly smaller lung volume in the vitamin D-deficient mice compared with controls but no difference in either surface area or septal thickness between the groups. However, the number of alveoli was lower in the vitamin D-deficient group of female mice but not in the male mice.

In a study from 2014, Mandell et al. (59) examined the effects of vitamin D on neonatal rat pup survival after antenatal endotoxin injection and on isolated endothelial and ATII cells. Pregnant Sprague-Dawley rats received intra-amniotic injection of 2.195 IU/kg endotoxin. The animals were assigned to a saline control (50 μL), endotoxin alone (10 μg endotoxin/50 μL saline), or endotoxin + vitamin D (50 pg vitamin D/50 μL saline). Cesarean sections were performed on day 22 of gestation. Newborn pups that were exposed to endotoxin alone...
received daily intraperitoneal injections of vitamin D (1 ng/g) or saline for 14 days. Oxygen saturation was measured at delivery, and survival was monitored daily throughout the observation period. Survival rate was calculated as the number of survived pups divided by the number of placental sacs that received intra-amniotic injections.

Antenatal vitamin D treatment improved oxygenation (78% vs. 87%) and survival (84% vs. 57%) significantly after endotoxin exposure compared with endotoxin alone. Furthermore, the study also included a description of the alveolar growth. Fetal sheep ATII cells were isolated, and the viability of the ATII cells was assayed using Trypan blue exclusion and counted on a hemacytometer (viability >90%). The study concluded that vitamin D has a proliferative and protective effect on fetal ATII cells and suggested that early vitamin D therapy might be a potential strategy for reducing the risk of acute respiratory distress.

In the most recent study from 2014, Yurt et al. (107) studied the effects of vitamin D deficiency on lung molecular and structural alterations in rat pups. Sprague-Dawley dams were assigned to four different dietary groups 4 wk before mating: 0, 250, 500, or 1,000 IU/kg cholecalciferol. All diets contained 4.5 g/kg calcium. The assigned dietary regimens were continued throughout pregnancy and lactation until the pups were killed. At postnatal day 21, pups were anesthetized and sedated, and their lung function was determined using whole-body plethysmography. Before death, trachea was excised en bloc and prepared for determination of tracheal contractility. The total pulmonary compliance was not significantly different in the four groups, but the tracheal contractility response to acetylcholine showed highest contractility in vitamin D-deficient and 1,000 IU/kg cholecalciferol-supplemented animals compared with 500 IU/kg cholecalciferol-supplemented animals. Serum samples were collected at death. 25(OH)D levels were measured by the electrochemiluminescence technique, and alkaline phosphatase and calcium levels were measured using a Roche Cobas autoanalyzer spectrophotometric. Whereas serum levels of 25(OH)D were lowest in the no-cholecalciferol group, a dose-dependent increase was seen in the circulating levels of 25(OH)D in the supplemented groups. Serum levels of calcium were unaffected by both vitamin D deficiency and supplemented cholecalciferol levels. Similarly, the levels of alkaline phosphatase were unaffected except in the 1,000 IU/kg cholecalciferol-supplemented group, where a significant decrease was seen. Lungs were removed and either flash-frozen in liquid nitrogen or inflated in situ with paraformaldehyde in phosphate buffer at a standard inflation pressure of 20 cmH2O. Immunoblot analysis and immunofluorescence staining were used to examine alveolar epithelial-mesenchymal interactions, and lung morphology was visualized by stereology. Perinatal vitamin D deficiency altered alveolar epithelial-mesenchymal signaling. Protein levels of mesenchymal lineage markers (PPAR-γ and CCAAT/enhancer binding protein) were lowest in the vitamin D-deficient animals, increasing in the 250 IU/kg- and 500 IU/kg-supplemented animals. Supplementation with 1,000 IU/kg cholecalciferol had no significant effect. In contrast, protein levels of myogenic mesenchymal markers (fibronectin and calponin) were highest in the vitamin D-deficient animals and significantly inhibited in the supplemented groups. The level of surfactant protein C was lowest in the vitamin D-deficient animals compared with the supplemented groups. Morphometrically, the radial alveolar count was significantly decreased, and the mean linear intercept was significantly increased in the vitamin D-deficient group compared with the supplemented groups. Furthermore a dose-dependent increase in choline incorporation into saturated phosphatidylcholine and triolein uptake was shown, indicating that vitamin D deficiency inhibited both triglyceride uptake and de novo surfactant phospholipid synthesis. In conclusion, perinatal supplementation with 500 IU/kg cholecalciferol appears to block the altered airway contractility and alveolar epithelial-mesenchymal signaling caused by vitamin D deficiency.

Laboratory studies. The presented laboratory studies (71, 75, 79) were used an established human pulmonary adenocarcinoma-derived cell line (NCI-H441) to study the impact of 1,25(OH)2D3 on the synthesis of surfactant, the 1,25(OH)2D3 binding capacity of the human ATII cells, and the effect of 1,25(OH)2D3 on fructose 1,6-bisphosphatase (F1,6-BP) gene expression.

In 2002, Rehan et al. (79) found that human ATII cells were able to convert 1,25(OH)2D3 into its metabolite 1,25(OH)2D3-3-epi-D3. 1,25(OH)2D3-3-epi-D3 is almost equipotent to 1,25(OH)2D3 regarding inhibition of keratinocyte proliferation and suppression of parathyroid hormone secretion from the parathyroid cells. NCI-H441 cells were subcultured and incubated with 1,25(OH)2D3 (1 μM), and the metabolites were visualized after HPLC analysis by gas chromatography mass spectrometry. To study the effect of both vitamin D compounds on the synthesis of surfactoproteins, NCI-H441 cells were incubated with either 1,25(OH)2D3 (10−9 M) or 1,25(OH)2D3-3-epi-D3 (10−9 M) and prepared for liquid scintillation spectrometry. The effects of both vitamin D compounds on the expression of SP-B were visualized by both Western blot analysis and RT-PCR after incubation with either 1,25(OH)2D3 (10−9 M) or 1,25(OH)2D3-3-epi-D3 (10−9 M). Both 1,25(OH)2D3 and 1,25(OH)2D3-3-epi-D3 were found to be significant stimulators of the synthesis of surfactant phospholipids in ATII cells. Furthermore, 1,25(OH)2D3-3-epi-D3 increased the SP-B mRNA gene expression and the synthesis of SP-B in human ATII cells.

Nguyen et al. (71) demonstrated in 2004 a 1.4-fold increase in the ability of ATII cytosol to bind 1,25(OH)2D3 in human ATII cells. Human NCI-H441 type II cells were incubated in the presence of 1,25(OH)2D3 (10−8 M) or ethanol in the absence or presence of radioinert hormone, and the binding capacity of the ATII cells was estimated. The cells were also used to study the expression of F1,6-BP, a regulatory enzyme in glucose synthesis and degradation, by Northern blot analysis. The F1,6-BP mRNA expression was increased by incubation with 1,25(OH)2D3 in the same dose as used in the binding study. The authors suggested that activation of the F1,6-BP enzyme might play a role in the 1,25(OH)2D3 action on surfactant synthesis via the gluconeogenesis pathway.

One year later in 2005, Phokela et al. (75) investigated the effects of 1,25(OH)2D3 on NCI-H441 cells, human fetal lung tissue in organ culture, and isolated ATII cells in primary culture. To study the expression of VDR human fetal lung and primary ATII, cells were incubated in the absence or presence of 1,25(OH)2D3 (10−7 M), and lysates were analyzed by Western immunoblot analysis. VDR was barely detectable in the absence of 1,25(OH)2D3 but increased dramatically in the presence of the hormone. Expression of SP-A was visualized

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by Northern blot and Western blot analysis in human fetal lung, primary ATII cells, and NCI-441 cells after incubation with various concentrations of 1,25(OH)2D3 (10−10 M or 10−7 M) in the absence or presence of dibutyryl cyclic AMP. Expression of SP-B was studied in human fetal lung and primary ATII cells and SP-C only in human fetal lung after the same procedure as described above. In general, 1,25(OH)2D3 decreased the expression of SP-A mRNA in human fetal lung tissue and reduced SP-A mRNA and protein levels in isolated ATII cells. No significant effect of 1,25(OH)2D3 on SP-B and SP-C mRNA levels was observed in human fetal lung tissue, but the levels of both SP-B mRNA and SP-B protein in ATII cells were increased in the presence of 1,25(OH)2D3.

Discussion

The evidence of an impact of vitamin D on human fetal and neonatal lung diseases is sparse. Our systematic search identified only one small human RCT and two observational studies, of which only one had adjusted the association analysis for confounders. Regarding lung development and maturation, several studies in rodents showed a positive effect of vitamin D on the proliferation of ATII cells and fibroblasts, surfactant synthesis, and upregulation of VDR in the lungs. These findings were supported by laboratory studies.

Human studies. The RCT of Backström et al. (5) showed a decreased need of ventilation and a trend toward a shorter duration of oxygen supplementation in the high-dose vitamin D group, implying an improved lung function. The RCT was not designed primarily to investigate the effect of vitamin D on mechanical ventilation days or days with oxygen supplementation, and no statistical power calculation was performed for the association. An increased serum 25(OH)D level in the high-dose vitamin D group was not documented until 6 wk of age (~42 days). However, the number of days on mechanical ventilation ranged up to 50 days and 60 days in the low- and high-dose vitamin D group, respectively, which was within the time period of documented increased serum 25(OH)D levels. A decreased need of mechanical ventilation is an important finding, as even modern invasive ventilator treatment for preterm neonates is associated with an increased risk of complications including BPD (67, 99). One other RCT has reported on a lower risk of preterm birth in mothers with resultant higher 25(OH)D, adjusted for race, but gave no lung disease data (100), whereas another smaller RCT of smaller size with later gestational age at enrollment found no difference in gestational age at delivery (43).

Only one identified observational study reported on RDS (4). An increased risk of RDS was found for 25(OH)D below 25 nmol/l in univariate analysis, which has sparse value given the lack of confounder control. Established risk factors for RDS include low gestational age, low birth weight, lack of antenatal glucocorticoids, race, genetic polymorphisms, male sex, multiparity, cesarean section, and low APGAR score (2, 22a, 37, 40, 51, 92). The impact of latitude or seasonal variation, as a marker of sun exposure and hence 25(OH)D variation, has, to our knowledge, only been reported in one Norwegian study, where the incidence of RDS was slightly increased during the autumn (78), which does not comply with the usual nadir of 25(OH)D in pregnancy during March-April (3).

The other identified observational study (50) concluded that the VDR FokI polymorphism was associated with an increased adjusted risk of BPD. However, the association was not detectable when controlling for the very important confounders, gestational age and birth weight. Although genetic polymorphisms are of interest and may especially be of importance between races, vitamin D-related polymorphisms identified by genome-wide association studies are believed to have less impact compared with environmental vitamin D exposures (9).

Established risk factors of BPD in human preterm neonates include lower gestational age and birth weight, assisted ventilation, oxidative stress, infection, persistent ductus arteriosus, and fluid overload (44, 88), and both postnatal steroids and retinoic acid are known to reduce the risk of BPD (21, 89).

Although human studies of high evidence level and high quality on vitamin D and RDS or BPD are missing, a Cochran meta-analysis of nine human vitamin A supplementation RCTs showed a reduced risk for oxygen requirement at 1 mo of age, or the combined outcome death at 1 mo or oxygen requirement at 36 wk postmenstrual age, in preterm neonates with birth weight ≤1,500 g or gestational age <32 wk gestation (21). Because vitamin D acts through the VDR on the same VDREs as the RXR of vitamin A, a similar effect of vitamin D could be plausible although yet not proven in humans. Although vitamin A deficiency is prevalent in sub-Saharan Africa, vitamin D deficiency is a more widespread global phenomena in pregnancy, especially in countries without official guidelines for vitamin D supplementation in pregnancy (18, 20). Moreover, the toxicity profile of vitamin A limits the use of this vitamin in nondeficiency countries.

On the case report evidence level, a subacute respiratory distress disorder associated with rickets was reported in 1977 in four 6–8-wk-old preterm infants born at gestational age 26–32 wk (31). Respiratory distress was not present at birth but developed gradually. By autopsy of one of the neonates, moderate uniform expansion of the alveoli, mild pulmonary congestion, and edema were the only abnormal lung findings. No evidence of BPD was reported, and 25(OH)D was not determined. Most probably, the rachitic respiratory distress was due to postnatal softening and fracture of the ribs as well as weakness of the respiratory muscles.

Hypovitaminosis D is frequent, not only in pregnancy, but also in preterm neonates. In Ireland, 78% of very preterm neonates born (gestational age <32 wk, or birth weight <1,500 g) had a serum 25(OH)D ≤50 nmol/l at 18 days of age despite vitamin D supplementation (65). Other studies have reported a mean cord blood 25(OH)D3 in preterm infants between 14.5 and 29.2 nmol/l (19, 22). This high prevalence implies that hypovitaminosis as a risk factor of RDS or BPD would be frequent and therefore detectable in larger cohort studies or matched case-control studies. Well-designed cohort or case-control studies on the impact of vitamin D on RDS or BPD in very and extremely preterm neonates are encouraged. To date, the sparse existing human data on the impact of vitamin D on RDS or BPD do, however, in conjunction support a hypothesis of a risk factor to be tested in RCTs.

Animal and laboratory studies: fetal lung development. The animal studies (23, 59–61, 68–71, 85, 108) demonstrated the presence of VDR in the lung primarily during the last period of gestation (days 19–22) when ATII cells differentiate, surfactant biosynthesis and secretion begin, and the glycogen content...
decreases. Incubation with 1,25(OH)2D3 induced an upregulation of the receptor and stimulated differentiation and proliferation in both fibroblasts and ATII cells.

According to Marin et al. (60, 61) 1,25(OH)2D3 accelerated the decrease in glycogen content and increased the synthesis of surfactant-related phospholipid and the secretion of surfactant by ATII cells isolated from fetal rat lung. Of clinical interest, dexamethasone had no additional effects on surfactant concentration in the presence of 1,25(OH)2D3, and dexamethasone alone did not induce surfactant exocytosis in contrast to 1,25(OH)2D3 (60, 61). Antenatal prevention of RDS with dexamethasone or other glucocorticoids to the pregnant mother is widely used and evidence based in threatening premature birth (13, 62). The animal data suggest that vitamin D may be an additional, if not alternative, option to glucocorticoids in the prevention of RDS in mothers with threatening premature birth.

Nguyen et al. (71) brought further evidence of a physiological role of 1,25(OH)2D3 by presenting an association between the expression of VDR and the different stages of lung maturation in fetal rats. Their findings of an increased F1,6BP mRNA expression by 1,25(OH)2D3 in human ATII cells indicates that activation of F1,6-BP may play a role in the actions of 1,25(OH)2D3 on surfactant synthesis through the pathway of gluconeogenesis. Besides the effects on gluconeogenesis, F1,6-BP might facilitate surfactant synthesis in the postnatal lung (30). The F1,6BP gene is known to bear VDRE, and both RA and 1,25(OH)2D3 induce F1,6BP gene expression in human differentiated monocytes (28).

Whereas Sakurai et al. (85) illustrated the ability of 1,25(OH)2D3 to stimulate the differentiation of both fibroblasts and ATII cells, others (53) have shown that RA also is a potential stimulator of lung maturation. Both 1,25(OH)2D3 and RA exert their effects by binding as ligands to their respective intracellular receptors, RARs and VDRs, which both can form heterodimers with RXR and thereby transduce the hormonal signal into altered gene transcriptional events. Furthermore, interactions between RA and vitamin D signaling pathways have been described in lungs (53) as well as in other organ systems (87).

The laboratory studies (71, 75, 79) supported that vitamin D has an effect on several physiological lung maturation processes in rodents. Rehan et al. (79) demonstrated the ability of vitamin D to stimulate the production and secretion of surfactant-related phospholipids in human ATII cells. Phokela et al. (75) demonstrated how 1,25(OH)2D3 increases SP-B mRNA expression in human ATII cells and reduces the expression of SP-A mRNA in human fetal lung tissue and isolated ATII cells. The increase in SP-B mRNA and reduction in SP-A mRNA caused by 1,25(OH)2D3 are similar to observations made in the presence of both glucocorticoids and RA (11, 75, 95), implying a complex regulation of fetal lung maturity and surfactant synthesis. Phokela et al. (75) also demonstrated how 1,25(OH)2D3 upregulates VDR in human fetal lung tissue and human isolated ATII cells, confirming findings by Nguyen et al. (71) in human ATII cells.

Taken together, the animal and laboratory studies have given detailed insights in the mechanistic actions through which vitamin D stimulates maturation of the fetal lung. Still, no studies have to our knowledge clarified the relationship between the production of 1,25(OH)2D3 in the fibroblasts, the upregulation of VDR, and the biosynthesis of surfactant in ATII cells during the late period of gestation. Further investigation of these relationships is warranted to elucidate the physiological role of 1,25(OH)2D3 in the fetal lung.

**Animal and laboratory studies: postnatal lung development.**

In the rat lung, the widespread expression of VDR decreases before term delivery and remains low the first days of life (69–71). VDR is present after birth in pulmonary macrophages as in almost all immune cells (55, 64, 68), and alveolar macrophages convert 25(OH)D to 1,25(OH)2D3 (23, 68, 80).

Besides the stimulation of differentiation and proliferation in ATII cells and fibroblasts, 1,25(OH)2D3 inhibits apoptosis in both cell types during the most active period of alveolarization and increases the alveolar count postnatally (23, 59, 60, 85). These findings suggest a local alveolar modulation of the postnatal alveolar growth by vitamin D.

Both Zosky et al. (108) and Mandell et al. (59) showed a link between vitamin D deficiency and altered postnatal lung development and maturation in rodents. Vitamin D improved oxygenation and survival after antenatal endotoxin exposure (59).

Even though the risk of bias analysis weakens the results by Sakurai et al. (85), others have found similar results (23, 59, 60, 71), which especially supports their postnatal findings. In the paper by Mandell et al. (59), blinding of the caretakers and investigators is not well described, but their findings are supported by Zosky et al. (108), whose risk of bias analysis does not raise any concerns.

Taken together, fetal and postnatal effects of vitamin D on lung maturation are well documented and suggest that vitamin D therapy may be a preventive or therapeutic option in preterm neonates against, not only RDS, but also BPD.

Future animal studies should be designed to identify optimal time windows and optimal doses for intervention with vitamin D treatment in vitamin D-depleted models at different stages in early pregnancy, late pregnancy, and in preterm offspring. However, the present knowledge from animal and laboratory data should encourage researchers to perform RCTs on the effect of vitamin D in the prevention and treatment of RDS and BPD. Population-based RCTs with vitamin D supplementation in pregnancy as a primary prevention must be very largely dimensioned to overcome the rareness of extreme or very premature delivery. Moreover, a control group without vitamin D supplementation would not be feasible in countries with recommended vitamin D supplementation. RCTs restricted to women with documented vitamin D deficiency or insufficiency must take the time delay of 25(OH)D determination into account.

RCTs targeted to women with threatening preterm birth or to the preterm neonates from the earliest possible time are more feasible options but with a short time interval between intervention and outcome, possibly requesting a high, single, or repeated vitamin D dose analogous to the well-established antenatal steroid therapy to women with threatening preterm delivery. The RCTs should investigate the effect of different vitamin D doses with determination of 25(OH)D to establish cut-off values for eventual effects and toxicity.

Animal data have shown an effect of vitamin D on respiratory airways. Foong et al. (27) showed a decreased expression of transforming growth factor (TGF)-β1 and TGF-β receptor I in vitamin D-depleted female mice fetuses on day E17.5. At the
postnatal age of 8 wk, airway resistance and airway smooth muscle mass were significantly increased in the large airways; lung volume, volume of parenchyma, and alveolar septa were smaller, and TGF-β levels were reduced in bronchoalveolar lavage fluid. Male mice only showed the latter change.

Yurt et al. (107) recently demonstrated both proximal and distal airway molecular and functional alterations caused by perinatal vitamin D deficiency and dose-dependent prevention of these alterations with cholecalciferol supplementation, indicating that vitamin D supplementation may have a clinical effect in preventing childhood asthma.

In keeping with the animal data, a recent human study (109) showed that lower maternal vitamin D concentrations in pregnancy were after confounder control associated with current wheeze (both sexes, questionnaire data, \( P = 0.05 \), current asthma (boys, doctor’s diagnosis, \( P = 0.04 \)), decreased functional vital capacity (FVC) \( Z \)-score (both sexes, \( P = 0.02 \), largely driven by decreased FVC in girls), a trend toward lower forced expiratory volume in 1 s (FEV1) \( Z \)-score at 6 yr (girls, \( P = 0.09 \)), and decreased FEV1/FVC \( Z \)-score in 14-yr-old girls (\( P = 0.05 \)). Whereas some other studies support an inverse relation between vitamin D status in pregnancy and offspring wheeze or asthma, others have found no associations, or U-shaped or direct associations, although many of these studies were of minor quality, e.g., in the diagnosis of childhood asthma (62, 63). Hopefully, ongoing RCTs like the Vitamin D Antenatal Asthma Reduction Trial (VDAART) (54) will provide high-quality, high-level evidence on this question.

Strengths of our study included the systematic review method without time limit in the search and the inclusion of human, animal, and laboratory data. Furthermore, a risk of bias analysis was performed.

Limitations included the paucity of high-evidence human data, which severely hampered conclusions for the clinical use of vitamin D. Extrapolation of rodent data to humans may be hampered by differences in lung development and maturation. The delayed surfactant synthesis and lack of prenatal alveolar phase in rodents provide suitable models for studies on lung diseases of the preterm but less suitable for studies on lung diseases of infants and children.

Conclusions on VDR expression were constrained by methodological issues on VDR detection by immunohistochemistry (69–71). New data show that the widely used VDR monoclonal antibody 9A7γ especially in rat tissue, not only binds to VDR, but also possesses nonspecific interactions with unidentified proteins (102, 103). On the basis of a parallel comparison of a large selection of VDR antibodies, the mouse monoclonal antibody D-6 possesses the highest specificity, sensitivity, and versatility. The antibody is capable of binding VDRs from human, monkey, pig, chicken, rat, and mouse and can be used for multiple immunoassays (102). Future studies on the VDR expression should use a more specific VDR antibody, e.g., D-6, which is highly sensitive, specific, and versatile.

Conclusion. Our systematic review on human data showed sparse evidence, allowing no conclusions on the potential role of vitamin D in the prevention or treatment of RDS or BPD in preterm neonates. Animal and laboratory data showed substantial evidence of multiple physiological actions through which vitamin D stimulates maturation of the fetal lung including ATII cell maturation and the alveolarization. These data give support to a hypothesis of vitamin D deficiency or insufficiency as a frequent, modifiable risk factor of RDS and BPD, which should be investigated in cohorts or case-control studies and tested in RCTs on pregnant women, especially with threatening preterm delivery, or in the preterm neonates themselves. Moreover, the effect of vitamin D on RDS or BPD in preterm neonates without vitamin D deficiency or insufficiency and potential adverse effects of high doses, including long-term outcomes as asthma, should be specifically addressed. Experimental and human studies should aim to identify optimal time windows, vitamin D doses, and cut-off levels for 25(OH)D for the intervention against RDS, BPD, and later respiratory outcomes.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: S.L. and H.T.C. conception and design of research; S.L. and H.T.C. analyzed data; S.L. interpreted results of experiments; S.L. drafted manuscript; S.L., G.L.S., S.S.B.-N., and H.T.C. edited and revised manuscript; S.L., G.L.S., S.S.B.-N., and H.T.C. approved final version of manuscript.

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