

# Pulmonary Fluid Balance in the Human Newborn Infant

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## Key Words

Epithelial sodium channels · Sodium transport · Lung fluid · Epithelial sodium channel

## Abstract

At birth, the infant's lungs must be cleared of fetal lung fluid. This process is mediated through the activation of airway epithelial sodium channels (ENaC). In animals, ENaC is considered crucial for postnatal pulmonary adaptation. In humans, postnatal ENaC expression is gestational age dependent and its activity, measured as nasal potential difference, correlates with lung compliance. It is therefore likely that in the human newborn infant ENaC is also important for physiologic postnatal adaptation. Low pulmonary expression or activity of ENaC in the perinatal period may cause delayed clearance of lung fluid and thereby contribute to development of respiratory distress in both term and preterm infants.

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## Introduction

At birth, the newborn infant is instantly faced with the challenge of adaptation from a fluid-filled environment to breathing air. While most infants undergo this transformation rapidly, some encounter difficulties. Preterm infants typically develop respiratory distress syndrome (RDS), while near-term and term infants may present

with transient tachypnea of the newborn (TTN). Both entities may be associated with inefficient lung liquid clearance, that is the inability to clear the lung of perinatal fluid [1–4]. In addition, decreased ion transport may also contribute to the pathogenesis of bronchopulmonary dysplasia (BPD) [5–7]. However, there are only limited data available describing the association of lung fluid transport and neonatal respiratory distress in human infants.

## Secretion of Lung Fluid in the Fetus

Fluid is present in the lung lumen from early lung development, presumably beginning as early as the sixth week of gestation [8]. There is a difference in composition between fetal lung fluid and amniotic fluid or plasma [9]. In the fetal lamb lung the transport of chloride ions takes place against the existing electrochemical gradient [10]. After further studies in rat alveolar epithelial cells it was suggested that the epithelium secretes  $\text{Cl}^-$  which thus drives fluid secretion [11].

Secretion of fluid into the lung lumen results in increased intrapulmonary pressure. The closed vocal cords, larynx and nasopharynx constrict the outflow of lung fluid [12–14]; therefore, the intrathoracic pressure in fetal sheep is, in the third trimester, higher than the elastic pressure of the chest wall [15]. Because pressure in the fetal lung is crucial for keeping the developing pulmonary structures open, lung development is dependent on

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a certain amount of lung fluid. Pathological states such as oligohydramnios, in which the formation of normal intrapulmonary fluid pressure is inhibited, result in lungs of small volume or hypoplastic ones [16]. This can also be the result of pulmonary arterial occlusion, congenital diaphragmatic hernia, skeletal dysplasia or diaphragmatic paralysis [17–19], in all of which intrapulmonary pressure is lower than in the healthy fetus. With a surplus of fetal lung fluid, the lungs become larger than normal or hyperplastic [20–22]. From mid-term towards term, lung fluid secretion increases but it decreases significantly before labor [23, 24].

### Perinatal Switch

At birth, the newborn infant needs to commence respiration, so rapid removal of fetal lung fluid is essential. Early experiments on animals showed significant reduction in lung fluid content after birth [25, 26]. In the mature newborn rabbit, lung wet weight decreases substantially during the first 2 h [25] and continues to decrease up to 24 h after birth [27]. Fetal breathing movements may clear some of the fetal lung fluid several days prior to birth [23]. In addition, some transient passive movement of lung fluid through the epithelium may occur during the first hours after birth [28, 29]. Amiloride is a specific inhibitor of sodium channels and has served as a means of studying sodium transport [30]. The finding that amiloride, instilled into the trachea of newborn guinea pigs in the early postnatal period, causes respiratory distress [31] increased interest in the role of sodium channels in postnatal lung fluid clearance.

Of the ion channels on the apical surface of the airway epithelium, the amiloride-sensitive sodium channel (ENaC) has been found to be rate-limiting for the process of lung fluid absorption across the epithelium [3]. ENaC was first isolated from the colonic epithelium of rats and soon afterwards was shown to be expressed also in the kidney and the lung [32–35]. In airway epithelium, ENaC is composed of three subunits,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -, each formed of two transmembrane domains, an extracellular loop and short N- and C-termini [32, 34]. The three subunits expressed together produce a 100-fold channel activity in comparison with that of  $\alpha$ -ENaC alone [3, 34, 36]. In addition, in different epithelia several apical amiloride-sensitive channels have been characterized by selectivity for  $\text{Na}^+$  over  $\text{K}^+$  [35, 37–39].

The importance of ENaC for postnatal pulmonary adaptation was highlighted in animal experiments when

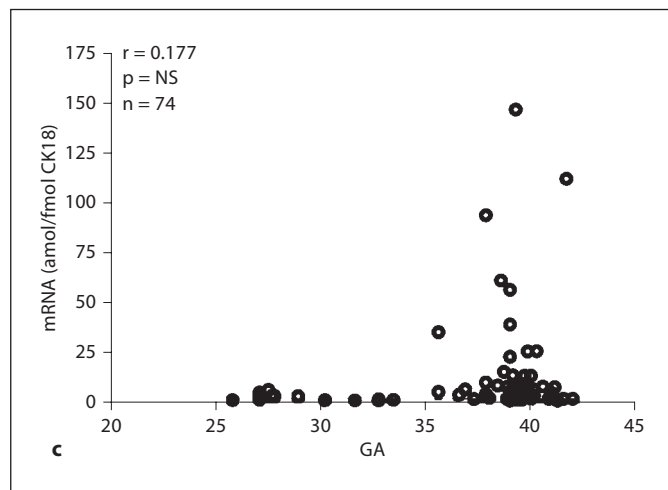
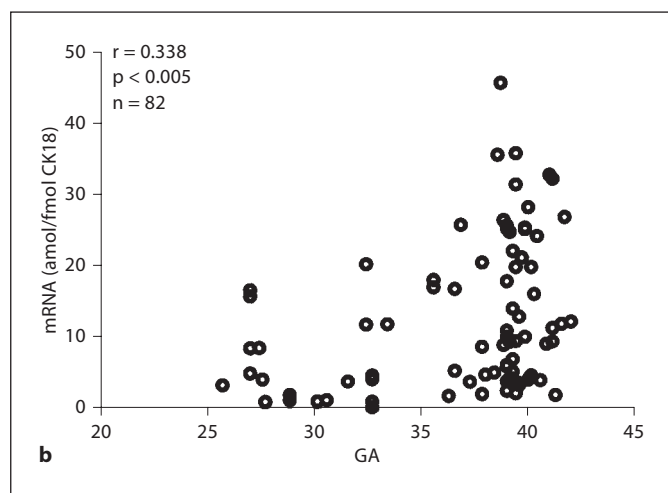
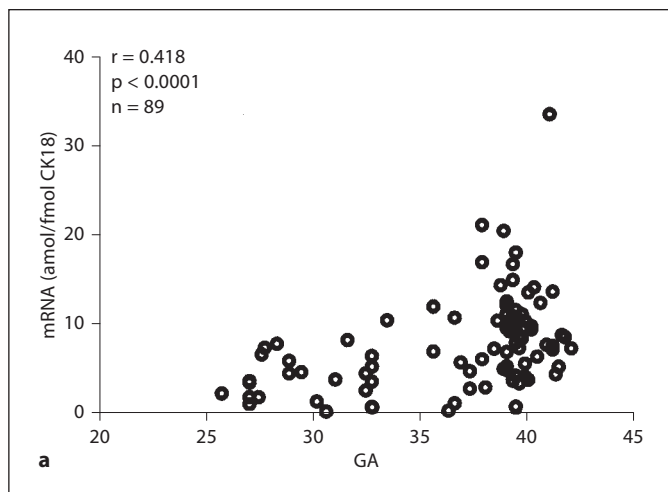
$\alpha$ -ENaC-knockout mice, unable to clear their lungs of perinatal fluid, died of respiratory insufficiency [3]. In rats, ENaC subunits are not expressed until late gestation [40]. In the human fetal lung, expression of ENaC subunits is present in the earliest stages of lung development [41], but the fetal expression of ENaC is lower than in the adult lung [35].

### Postnatal Pulmonary Adaptation in Human

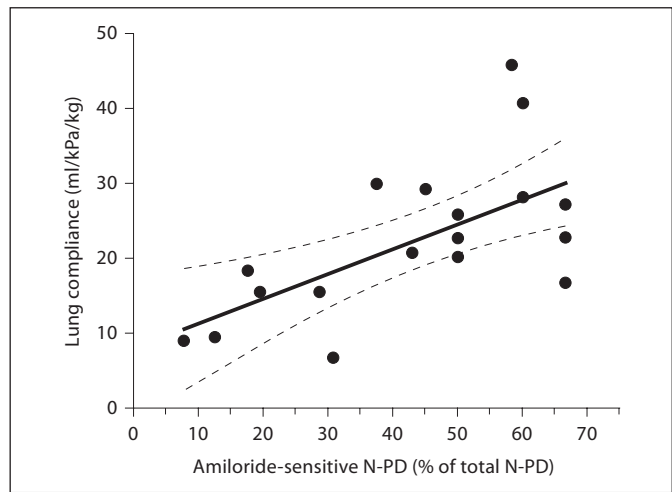
There are only a few studies on human postnatal pulmonary adaptation. These include studies mostly focusing on the upper airway epithelium: airway epithelial ENaC subunit expression analysis [42, 43] and the nasal transepithelial potential difference (N-PD), a surrogate measurement for ion transport developed for diagnosis of cystic fibrosis [44]. In conjunction with some of these studies, lung compliance has been measured.

### Epithelial Sodium Channel Subunit Expression in the Airway Epithelium

In the healthy newborn infant, studies on airway epithelial ENaC expression suggest a distinct expression profile for each subunit over a period of 48 h postnatally [45, 46]. Whereas the expression of the  $\alpha$ -subunit remains quite stable after birth, there is a significant decrease in the expressions of the  $\beta$ - and  $\gamma$ -subunits during this period [47]. In preterm infants the expression of ENaC subunits has a different profile with a significant decrease only in the expression of the  $\beta$ -subunit during the first 28 h postnatally [46]. In the newborn human infant, ENaC expression is dependent on gestational age [46] (fig. 1). It remains unclear what purpose the differences over time in subunit expressions serve. It is, however, apparent that in *in vivo* studies in  $\alpha$ -ENaC-knockout and  $\beta$ -ENaC-knockin rats there is an essential need for  $\alpha$ -ENaC expression for the clearance of lung fluid during postnatal pulmonary adaptation, and that low expression of  $\beta$ -ENaC impairs alveolar fluid clearance in the adult rat [3, 48]. In addition, in the rat the overexpression of  $\beta$ -ENaC induces cystic fibrosis-like symptoms [49]. Hence, the low expression of subunits in preterm infants during the first day of life may well contribute to respiratory distress in these infants.



**Fig. 1.** Correlation between ENaC subunit expression and gestational age in airway epithelium in newborn infants 1–5 h after birth: **a**  $\alpha$ -ENaC, **b**  $\beta$ -ENaC, and **c**  $\gamma$ -ENaC. CK18 = Cytokeratin 18; NS = not significant [reproduced from 46, with permission of APP].

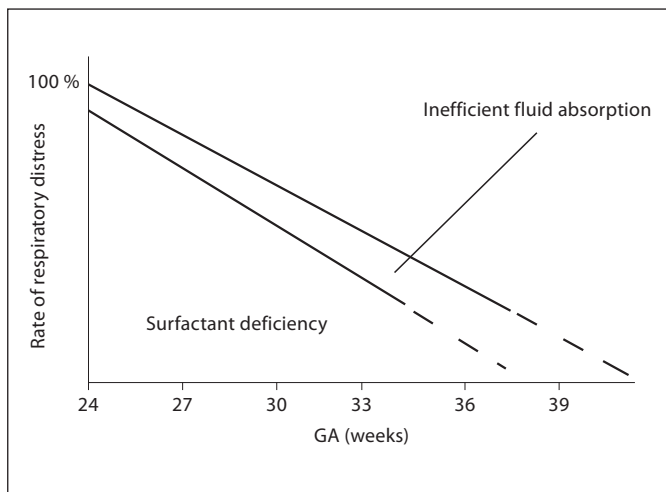


**Fig. 2.** Postnatal amiloride-sensitive sodium transport of nasal epithelium at 1–4 h and static lung compliance at 21–48 h in 20 newborn infants. Sodium transport is expressed as the percent inhibition of potential difference caused by amiloride. Regression line ( $r^2 = 0.40$ ,  $p < 0.003$ ) and 95% confidence intervals are shown [reprinted from 53, with permission of Elsevier].

### Nasal Transepithelial Potential Difference

ENaC can be blocked with amiloride. In the nasal epithelium of the preterm newborn infant, amiloride-sensitive N-PD, a correlate for ENaC activity, has been shown to correlate with gestational age [50]. Preterm infants have significantly lower amiloride-sensitive N-PD than term infants [51, 52], suggesting impaired airway epithelial ion transport and inability to clear the lung of perinatal fluid. In TTN, there is a lower amiloride-sensitive N-PD than in healthy newborn infants [2]. N-PD measurements have also been performed on preterm infants with and without BPD, showing that at 29 days of age infants with BPD have significantly lower N-PD than those without BPD [50]. Accordingly, BPD may be associated with a prolonged impairment of airway ion transport.

In healthy term infants, lung compliance increases in the first few postnatal days [47]. In newborn term infants there is a positive correlation between amiloride-sensitive N-PD measured at 1–5 h and lung compliance at 21–48 h after birth [47, 53] (fig. 2). In healthy term newborn infants there is no significant correlation between airway epithelial ENaC expression and N-PD or lung compliance [47]. However, it is possible that in healthy term newborns the post-transcriptional mechanisms are more important in regulating epithelial ion transport than in affected infants where transcription may be rapidly increased.



**Fig. 3.** Relative contribution of surfactant deficiency and insufficient fluid absorption to neonatal respiratory distress.

### Enhancing Lung Fluid Transport

Glucocorticoids have been shown to induce  $\alpha$ -ENaC transcription through the activation of a glucocorticoid-responsive element in its 5'-flanking region [54, 55]. All three ENaC subunits are upregulated by glucocorticoids in human fetal lung explant cultures [56]. Also, glucocorticoids increase ENaC trafficking and retention in the membrane with the help of serum- and glucocorticoid-regulated kinase (SGK) [57]. An increase in SGK expression is linked to an increased expression of  $\alpha$ -ENaC [58]. SGK phosphorylates developmentally downregulate protein 4-2 (Nedd4-2) [59] which inhibits the binding of Nedd4-2 to ENaC and therefore reduces ENaC degradation [60]. Silencing Nedd4-2 increases lung ENaC expression in newborn rats [61].

Antenatal corticosteroids have been shown to decrease the risk [62] and incidence of RDS [63, 64]. While boosting surfactant synthesis and secretion the effect may, in part, also be mediated by more efficient ENaC activity and thus lung fluid removal. Importantly, the effect of increased rate of lung fluid clearance was demonstrated in term and near-term infants born by elective cesarean section to mothers who had received antenatal betamethasone [65]. The infants also had a decreased incidence of neonatal respiratory distress. Because RDS, characterized by surfactant deficiency, is uncommon in term infants [66], it is possible that the effect of betamethasone at term is predominately mediated by increased ion trans-

port and thus lung fluid removal [65]. Finally, dexamethasone treatment given postnatally to preterm infants with BPD and several weeks of assisted ventilation increases the expression of all ENaC subunit mRNAs [45] coinciding with weaning of the infants from artificial ventilation during treatment. These observations suggest that glucocorticoid treatment indeed has a beneficial effect on airway epithelial ion transport and lung liquid absorption.

In view of these studies, performed in animals and humans alike, it seems likely that while surfactant deficiency plays a major role in the respiratory distress of preterm infants, it may, in addition, be affected also by the inability to clear the lung of perinatal fluid [31, 45, 52]. However, in term newborn infants it is likely that the main entity causing respiratory distress is, in fact, insufficient fluid clearance from the lung lumen [2, 65]. We hypothesize that the proportion of surfactant deficiency as a causative factor for neonatal respiratory distress diminishes with gestational age while the inability to clear the lung of perinatal fluid remains fairly constant (fig. 3).

### Conclusion

There are few studies on human neonates focusing on ion transport. The data so far suggest that ion transport activity correlates with lung compliance and, in addition, that insufficient ion transport in airway epithelium may be a contributing factor in neonatal lung disease. It is possible that the induction of channel activity by, for example, glucocorticoids could attenuate the disease.

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