The Effect of Continuous Positive Airway Pressure in a Mouse Model of Hyperoxic Neonatal Lung Injury

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Key Words

Hyperoxia · Continuous positive airway pressure ·
Bronchopulmonary dysplasia · Alveolar development ·
Pulmonary mechanics

Abstract

Background: Continuous positive airway pressure (CPAP) and supplemental oxygen have become the mainstay of neonatal respiratory support in preterm infants. Although oxygen therapy is associated with respiratory morbidities including bronchopulmonary dysplasia (BPD), the long-term effects of CPAP on lung function are largely unknown. We used a hyperoxia-induced mouse model of BPD to explore the effects of daily CPAP in the first week of life on later respiratory system mechanics. **Objective:** We wanted to test the hypothesis that daily CPAP in a newborn-mouse model of BPD improves longer-term respiratory mechanics. **Methods:** Mouse pups from C57BL/6 pregnant dams were exposed to room air (RA) or hyperoxia (50% O₂, 24 h/day) for the first postnatal week with or without exposure to daily CPAP (6 cm H₂O, 3 h/day). Respiratory system resistance (Rrs) and compliance (Crs) were measured following a sub-

sequent 2-week period of RA recovery. Additional measurements included radial alveolar and macrophage counts. **Results:** Mice exposed to hyperoxia had significantly elevated Rrs, decreased Crs, reduced alveolarization and increased macrophage counts at 3 weeks when compared to RA-treated mice. Daily CPAP treatment significantly improved Rrs, Crs and alveolarization and decreased lung macrophage infiltration in the hyperoxia-exposed pups. **Conclusions:** We have demonstrated that daily CPAP had a longer-term benefit on baseline respiratory system mechanics in a neonatal mouse model of BPD. We speculate that this beneficial effect of CPAP was the consequence of a decrease in the inflammatory response and resultant alveolar injury associated with hyperoxic lung injury in newborns.

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Introduction

Bronchopulmonary dysplasia (BPD) is a chronic respiratory disease that affects over 30% of preterm infants born at <30 weeks gestational age [1, 2]. The development of BPD results from neonatal lung injury which is thought

to be caused primarily by supplemental oxygen therapy and mechanical ventilation, and it remains a major form of respiratory morbidity in individuals who were born preterm [3, 4]. Although lung growth and remodeling may improve over time, lung function abnormalities typically comprising airway obstruction and wheezing disorders remain throughout childhood and adolescence, and are also observed in preterm infants without the diagnosis of BPD [4–7]. As a consequence, many preventive and therapeutic interventions have been investigated in this population, but the prevalence of respiratory morbidity remains high.

There is no current consensus on the best respiratory support strategy in preterm patients at risk of developing BPD, but there has been a substantial increase in the use of continuous positive airway pressure (CPAP) as the primary respiratory modality to avoid intubation and thereby decrease potential lung injury being caused by mechanical ventilation [8]. Experimental animal studies also demonstrate the benefit of noninvasive respiratory support versus mechanical ventilation in promoting alveolarization and preventing inflammation [9, 10]. CPAP given to young ferrets for 2 weeks demonstrated accelerated lung growth and a 40% increase in total lung capacity [11]. The authors of that study suggested that mechanical strain, i.e. CPAP, improves lung growth by remodeling the lung parenchyma. To our knowledge, there are no available data on the longer-term effects of CPAP on respiratory system mechanics in a neonatal animal model of hyperoxia-induced lung injury.

Initial experiments in neonatal mice using 85% O₂ showed alveolar simplification and lung dysfunction, creating a lesion similar to human BPD [12]. Recent experiments in a rodent model showed that even moderate amounts of oxygen can result in long-lasting impairment of the lung structure and function [13–15]. Therefore, the purpose of this study was to create a BPD phenotype based on exposure to moderate levels of hyperoxia, in order to test the hypothesis that daily CPAP would elicit long-lasting beneficial effects on respiratory system mechanics.

Materials and Methods

Animal Model and Exposure

Animal protocols were approved by the Institutional Animal Care and Use Committee at Case Western Reserve University. Timed-pregnant C57BL/6 mice (Charles River) were purchased and housed in an accredited animal facility at the university. Mouse pups from pregnant dams were pooled within 24 h of birth

and randomly assigned into treatment groups according to the exposure to: (1) room air (RA), (2) RA with daily CPAP (+6 cm H₂O, 3 h/day on days 1–7), (3) continuous hyperoxia (50% O₂, 24 h/day for 7 days), and (4) hyperoxia (50%) with CPAP (+6 cm H₂O, 3 h/day for 7 days). Oxygen exposures and CPAP were initiated on postnatal day 1 (P1) and ended on P7. Animals were maintained on standard 12-hour light-dark cycles with ad libitum standard food and water. Hyperoxia-exposed animals were placed in a 38-liter Plexiglas chamber with a continuous flow of 50% O₂ (4 liters/min) for 7 days. Hyperoxia was achieved by mixing air and 100% O₂ at appropriate amounts to achieve the desired level of O₂, which was monitored daily (MiniOX I, MSA Medical). Hyperoxia-exposed mice that were designated to receive CPAP were removed from the hyperoxia chamber and a mask that delivered 50% O₂ was immediately fitted, and CPAP was administered. CPAP was applied by passing humidified gas (RA or 50% O₂, depending on the treatment group) through a custom-made mask fitted to the face of the mouse as previously described [16]. This mask was constructed from a 3-ml syringe that had been cut in half to accommodate the entire face of the animal. The ‘open’ end of the syringe allowed for positioning of a latex sleeve containing a hole large enough to accommodate the face. The latex sleeve was constructed from the fingertip of latex gloves. The level of CPAP was monitored using a manometer connected directly to the mask, and could be finely adjusted by altering the size of a leak using an adjustable valve located between the mask and the manometer. At the end of the daily CPAP session, the mice were returned to the mother and the hyperoxic group was placed back inside the hyperoxia chamber. CPAP was restricted to 2 h on the first day to minimize the time separated from the nursing dam. On the following 6 days, CPAP duration was increased to 3 h/day. Control animals experienced the same conditions, i.e. mask, humidified air, heat-pad and 3 h of separation from the dam, but without CPAP. On rare occasions, the animal was able to maneuver its way out of the mask, which was then repositioned immediately, as the animals were under constant observation. Due to the small size of the neonatal pups, precise measurement of pressures to which various components of the airways were exposed could not be measured. On day 7, the mice were returned to the nursing dam in RA and allowed to develop normally for an additional 14 days. At P21, the animals were weighed before obtaining measurements of respiratory mechanics or tissue preparation. There was no significant difference between average body weights at 3 weeks and there was an approximately equal distribution of females and males between groups.

Pulmonary Function Tests

Mice from the 4 exposure groups (RA: n = 10, RA and CPAP: n = 7, 50% O₂: n = 12 and 50% O₂ and CPAP: n = 7) were anesthetized at P21 with a mixture of intraperitoneal ketamine (200 mg/kg) and xylazine (20 mg/kg). Anesthetized mice were placed supine on a heated surgical table, tracheostomized via a 19-gauge blunt-tip cannula and ventilated (flexiVent, SCIREQ, Montreal, Canada) at a tidal volume of 10 ml/kg, 150 breaths/min, a PEEP value of 3 cm H₂O and FiO₂ of 50%. Animals were then paralyzed with an intraperitoneal injection of pancuronium bromide (10 mg/kg), and respiratory system resistance (Rrs) and compliance (Crs) were calculated with the flexiVent software using a 1.2-second, 2.5-Hz, single-frequency, forced-oscillation maneuver [17].

Fig. 1. Effect of exposure to CPAP on respiratory system mechanics in normoxic (RA) and hyperoxic mouse pups. **a** Hyperoxic exposure increased Rrs and this effect was reversed by CPAP in hyperoxic pups. Hyperoxic pups exposed to CPAP also had lower Rrs than both RA control groups. **b** Hyperoxic exposure decreased Crs when compared to both RA and hyperoxic groups treated with CPAP. * $p < 0.05$.

Lung Alveolarization Assessment

In order to assess lung morphology, additional mice (RA: $n = 10$, RA and CPAP: $n = 11$, 50% O₂: $n = 7$ and 50% O₂ and CPAP: $n = 7$) were removed from litters at P21 and euthanized with an intraperitoneal injection of ketamine/xylazine. The trachea was cannulated and lungs were inflation-fixed (25 cm H₂O) for 10 min with 10% neutral-buffered formalin. The left lung was removed, postfixed for 2 days at 4°C and then paraffin-embedded. Sections were stained with hematoxylin and eosin, and alveolarization was assessed by performing radial alveolar counts (RAC). Images of each section were captured with a magnified digital camera through a Leica microscope. RAC was determined by counting the number of alveolar septa transected by a perpendicular line drawn from the terminal bronchiole to the nearest connective tissue septum [18], and was averaged from 8–10 sections per animal from each of the 4 treatment groups. We also quantified alveolar-to-airway attachments concerning 3–7 randomly selected airways from each animal (5–6 animals/group). The points where alveolar walls are radially attached to the outer wall of the airways were counted as previously described [14]. They were quantified as the number of attachment points around the margin of the airways in relation to the basement membrane perimeter (P_{bm}). Only complete airways without alveolar ducts were analyzed.

Mac3 Immunohistochemistry

Lungs from the 4 groups (RA: $n = 4$, RA and CPAP: $n = 4$, 50% O₂: $n = 6$ and 50% O₂ and CPAP: $n = 6$) were inflation-fixed (25 cm H₂O) for 10 min with 10% neutral-buffered formalin. The 5- μ m paraffin-embedded tissue was treated with 3% H₂O₂ after antigen retrieval. Lung sections were incubated for 24 h at 4°C with rat anti-mouse Mac3 monoclonal antibody (1:500; BD Pharmin-

gen, San José, Calif., USA). The secondary antibody was biotinylated goat anti-rat (1:500; Jackson ImmunoResearch, West Grove, Pa., USA), followed by ABC reagent incubation and visualization by diaminobenzidine (Vectastain, Vector Laboratories, Burlingame, Calif., USA). Finally, lung sections were dehydrated and mounted with permount. Primary antibody was omitted for negative controls. All images were obtained using a Rolera XR CCD camera (Q-Imaging, Canada) mounted on a Leica DMLB microscope (Leica Microsystems, Germany). Images were analyzed using research-based digital image analysis software (ImagePro Plus 7.0, Media Cybernetics, Silver Spring, Md., USA). Numbers of labeled macrophages were counted in 5 random fields at $\times 20$ magnification from 4–6 animals per group.

Statistical Analysis

Data are expressed as means $\pm$ standard error of the mean. Rrs and Crs, RAC, alveolar-to-airway attachments and macrophage counts were compared using a one-way analysis of variance with Student-Newman-Keuls method. Statistical significance was defined as $p < 0.05$.

Results

Pulmonary Function Tests

The Rrs and Crs of the experimental groups were compared. CPAP alone had no effect on the Rrs when compared to RA (fig. 1a). In contrast, hyperoxia exposure compared to RA increased the Rrs (1.35 ± 0.17 vs. 1.19 ± 0.12 , respectively, $p < 0.05$). In the hyperoxia groups, the addi-

Fig. 2. a RAC from the 4 groups of pups are summarized. **b** Representative images. Hyperoxic exposure decreased RAC and this decrease was reversed when hyperoxic pups were exposed to CPAP. * $p < 0.05$, $p = 0.05$.

tion of CPAP attenuated the increase in Rrs observed with hyperoxia alone (1.35 ± 0.17 vs. 1.03 ± 0.14 cm H₂O/ml $\times$ s, hyperoxia vs. hyperoxia + CPAP, $p < 0.005$). Levels of Rrs in the hyperoxia CPAP pups were also significantly below the levels in both RA groups ($p < 0.05$). As with Rrs, CPAP alone, compared to RA, had no effect on Crs (fig. 1b). Hyperoxia exposure induced a decrease in Crs that did not reach statistical significance compared to RA exposure ($p = 0.053$), but was significantly lower than in the pups exposed to CPAP alone (0.015 ± 0.003 vs. 0.019 ± 0.005 ml/cm H₂O, hyperoxia vs. RA + CPAP, $p = 0.04$). In the

hyperoxia groups, the addition of CPAP increased Crs (0.015 ± 0.003 vs. 0.020 ± 0.005 ml/cm H₂O, hyperoxia vs. hyperoxia + CPAP, $p < 0.05$). Levels of Crs in the hyperoxia + CPAP pups were comparable to both the RA groups.

Lung Morphometrics

Alveolarization was assessed at 3 weeks of age via morphometric analysis of lung sections stained with hematoxylin and eosin. CPAP alone caused a borderline decrease in RAC in the RA pups (10.2 ± 1.03 vs. 9.15 ± 1.09 , $p = 0.05$; fig. 2). The hyperoxic pups demonstrated sig-

Fig. 3. Representative images of macrophages stained with Mac3 anti-macrophage antibodies from the 4 groups of pups (**b**) and a negative control (**c**) are shown together with summarized data (**a**). Hyperoxic exposure increased Mac3 counts in lung parenchyma when compared to both RA groups. This increase was prevented with CPAP exposure in the hyperoxic pups. * $p < 0.05$. The number of macrophages/high-power field (HPF) was counted. $\times 20$.

nificantly lower radial alveolar counts than the RA-exposed pups (8.07 ± 0.63 vs. 10.2 ± 1.03 , hyperoxia vs. RA, $p < 0.05$). In the hyperoxia groups, the addition of CPAP prevented the hyperoxia-induced reduction in RAC (8.07 ± 0.63 vs. 9.92 ± 0.92 , hyperoxia vs. hyperoxia + CPAP, $p < 0.05$). Data for alveolar-to-airway attachments are consistent with the RAC data, but they did not reach statistical significance (RA 26.4 ± 1.6 ; RA + CPAP 25.1 ± 1.6 ; hyperoxia 20.6 ± 1.5 ; hyperoxia + CPAP 22.4 ± 1.7 , $p = 0.07$).

Macrophage Counts

To assess inflammation, pulmonary macrophage numbers were counted on lung tissue sections from mice stained with Mac3 anti-macrophage antibodies (fig. 3). RA controls showed very few interstitial Mac3-positive cells as did those in the RA + CPAP group. Hyperoxia alone resulted in a significant increase in parenchymal cells that stained positive for Mac3 (11.82 ± 3.41) when compared to either RA controls (6.05 ± 0.60 , $p < 0.05$), RA + CPAP (6.7 ± 2.57 , $p < 0.05$) or the hyperoxia + CPAP group (8.06 ± 2.71 , $p < 0.05$).

Discussion

Hyperoxia Model

In this study, we employed a hyperoxia-exposed, immature rodent model to test the hypothesis that CPAP might have longer-term benefit for lung injury. Many of the commonly used animal models induce acute lung injury by increasing the amount of supplemental oxygen, exposing immature lungs to mechanical ventilation and/or inducing pre- or postnatal inflammation [19–21]. While the injurious effects of oxygen can be replicated in many species, the newborn mouse demonstrates stages in lung maturity similar to extremely preterm human infants. Murine alveolar development begins on P3 and saccular division is completed by P14 [22]. Neonatal rodents exposed to supplemental oxygen are also best-suited to characterize the longer-term effects of neonatal lung injury. As the oxygen concentrations used in many earlier animal models of BPD exceeded the level of oxygen supplementation currently applied to preterm infants, our model employed 50% oxygen to better reflect the modern clinical setting in which high FiO_2 is avoided whenever possible. Our results demonstrate that such modest exposure to 50% oxygen for 7 days causes impairment in respiratory function that is characteristic of BPD, similar to previous studies using small-animal

models exposed to higher oxygen concentrations [10, 20, 23]. It should be noted, however, that maturation of the murine immune system is a dynamic process which may not parallel the pre- and postnatal human maturational process and may not be in synchrony with lung development.

CPAP Model

Many preterm infants only require short periods, if any, of intubation and mechanical ventilation, but instead, they actually spend much longer periods on non-invasive support (i.e. CPAP). Therefore, there is a need for an animal model of BPD that simulates current clinical practice in the human preterm infant. We have previously reported the ability to successfully administer CPAP to a neonatal mouse [16]. This is the first report of in vivo respiratory function data following CPAP administration to a small rodent; longer-term follow-up experiments of respiratory function could be conducted with this model.

Protective Effects of CPAP

The results of this study demonstrate that daily CPAP protected both Rrs and Crs from the adverse effects of oxygen exposure. Previous studies on preterm lambs demonstrated that nasal ventilation (performed frequently), somewhat analogous to CPAP, appears to support normal alveolar development by preserving the balance between mesenchymal cell apoptosis and proliferation, when compared to the injurious effects of intermittent mandatory ventilation [10]. This is consistent with other studies on preterm lambs that demonstrated that CPAP or gentler modes of ventilation reduce key markers of lung injury and inflammation [24, 25]. In our study, alveolarization was preserved by CPAP in the hyperoxia pups with a resultant improvement in Crs.

In the pups exposed to CPAP in the absence of hyperoxia, there was a borderline decrease in RAC with no significant effect on baseline respiratory mechanics. There was also no clear decrease in alveolar attachments after CPAP exposure in the RA group, which could suggest that CPAP administration alone did not have a clearly defined effect on the lung parenchyma. Our observed decrease in RAC with hyperoxic exposure was associated with a trend towards a reduction in alveolar attachments. Fewer alveolar attachments have been observed in mature mice exposed to a higher level of postnatal hyperoxia than we used in our study [14]. We have no clear explanation for the significantly lower Rrs after hyperoxia + CPAP exposure compared to the RA controls. Our data on lung

morphology do not provide an explanation for this low Rrs after combined hyperoxia and CPAP exposure. We speculate that this combination has a selective effect on the airway structures; this requires further in vivo and in vitro study.

We have previously documented an increase in airway reactivity in an in vitro lung-slice model after neonatal mice were exposed to CPAP under normoxic conditions [16]. Unlike our in vivo study, the lung slices did not allow measurement of baseline respiratory mechanics. Our current data demonstrate that baseline resistance was not affected by prior CPAP in normoxic pups and the beneficial effect of CPAP was limited to our hyperoxia-exposed pups. The longer-term benefit on respiratory system mechanics was accompanied by a decrease in macrophage-mediated inflammation in the hyperoxia-exposed pups. A detailed analysis of the effect of CPAP on the time course of other inflammatory markers such as neutrophils and various cytokines will require future study on larger groups. However, from previous studies on neonatal rodents, it is anticipated that there would be a link between increased inflammation, measured by macrophage infiltration elicited by hyperoxia, and reduced RAC [19, 20]. Future studies employing our novel rodent model for CPAP administration might also provide clinical guidance for neonatologists who are confronted daily with the dilemma of when to wean infants off CPAP, but without understanding how this may alter future lung function.

Limitations

Although our daily mask applications with and without added CPAP were surprisingly well tolerated, we chose to only administer CPAP for 3 h daily, in order to minimally disrupt the pups and avoid longer periods away from maternal care. Even with such minimal exposure to CPAP, we found a protective effect for the hyperoxia-exposed mice. We selected only 1 recovery time point at P21 to assess our results; this was deliberately chosen as a marker for the impaired respiratory function of preterm infants that continues until early childhood [5, 6].

In conclusion, in our model of BPD, CPAP protected against longer-term oxygen-induced lung injury, as manifest by the improved respiratory system mechanics and alveolar morphology. We speculate that greater understanding of stretch-mediated molecular pathways may play an important role in our future understanding of the airway remodeling and alveolar simplification that are associated with longer-term respiratory morbidity in preterm infants.

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References

- 1 Jobe AH, Bancalari E: Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001; 163:1723–1729.
- 2 Baraldi E, Filippone M: Chronic lung disease after premature birth. *N Engl J Med* 2007;357: 1946–1955.
- 3 Greenough A: Bronchopulmonary dysplasia – long-term follow up. *Paediatr Respir Rev* 2006;7S:S189–S191.
- 4 Reyburn B, Martin RJ, Prakash YS, MacFarlane PM: Mechanisms of injury to the preterm lung and airway: implications for long-term pulmonary outcome. *Neonatology* 2012;101:345–352.
- 5 Fawke J, Lum S, Kirkby J, Hennessy E, Marlow N, Rowell V, Thomas S, Stocks J: Lung function and respiratory symptoms at 11 years in children born extremely preterm. *Am J Respir Crit Care Med* 2010;182:237–245.
- 6 Been JV, Lugtenberg MJ, Smets E, van Schayck CP, Kramer BW, Mommers M, Sheikh A: Preterm birth and childhood wheezing disorders: a systematic review and meta-analysis. *PLoS One* 2014;11:1–18.
- 7 Vollsaeter M, Roksund OD, Eide GE, Markestad T, Halvorsen T: Lung function after preterm birth: development from mid-childhood to adulthood. *Thorax* 2013;58:767–776.
- 8 Committee on Fetus and Newborn; American Academy of Pediatrics: Respiratory support in preterm infants at birth. *Pediatrics* 2014; 133:171–174.
- 9 Null DM, Alvord J, Leavitt W, Wint A, Dahl MJ, Presson AP, Lane RH, DiGeronimo RJ, Yoder BA, Albertine KH: High-frequency nasal ventilation for 21 days maintains gas exchange with lower respiratory pressures and promotes alveolarization in preterm lambs. *Pediatr Res* 2014;75:507–516.
- 10 Reyburn B, Li M, Metcalfe DB, Kroll NJ, Alvord J, Wint A, Dahl MJ, Sun J, Dong L, Wang ZM, Callaway C, McKnight RA, Moyer-Mileur L, Yoder BA, Null DM, Lane RH, Albertine KH: Nasal ventilation alters mesenchymal cell turnover and improves alveolarization in preterm lambs. *Am J Respir Crit Care Med* 2008;178:407–418.
- 11 Zhang S, Garbutt V, McBride JT: Strained-induced growth of the immature lung. *J Appl Physiol* 1996;81:1471–1476.
- 12 Warner BB, Stuart LA, Papes RA, Wispe JR: Functional and pathological effects of prolonged hyperoxia in neonatal mice. *Am J Physiol* 1998;275:L110–L117.
- 13 Wang H, Jafri A, Martin RJ, Nnanabu J, Farver C, Prakash YS, MacFarlane PM: Severity of neonatal hyperoxia determines structural and functional changes in developing mouse airway. *Am J Physiol Lung Cell Mol Physiol* 2014;307:L295–L301.
- 14 O'Reilly M, Harding R, Sozo F: Altered airways in aged mice following neonatal exposure to hyperoxic gas. *Neonatology* 2014;105: 39–45.

- 15 Onugha H, MacFarlane PM, Mayer CA, Abrah A, Jafri A, Martin RJ: Airway hyperreactivity is delayed after mild neonatal hyperoxic exposure. *Neonatology* 2015;108:65–72.
- 16 Mayer CA, Martin RJ, MacFarlane PM: Increased airway reactivity in a neonatal mouse model of continuous positive airway pressure. *Pediatr Res* 2015;78:145–151.
- 17 Shalaby KH, Gold LG, Schuessler TF, Martin JG, Robichaud A: Combined forced oscillation and forced expiration measurements in mice for the assessment of airway hyperresponsiveness. *Respir Res* 2010;11:82–94.
- 18 Cooney TP, Thurlbeck WM: The radial alveolar count method of Emery and Mithal: a reappraisal 2 – intrauterine and early postnatal lung growth. *Thorax* 1982;37:580–583.
- 19 Choi CW, Kim BI, Mason SN, Potts-Kant EN, Brahmajothi MV, Auten RL: Intra-amniotic LPS amplifies hyperoxia-induced airway hyperreactivity in neonatal rats. *Pediatr Res* 2013;74:11–18.
- 20 Velten M, Heyob KM, Rogers LK, Welty SE: Deficits in lung alveolarization and function after systemic maternal inflammation and neonatal hyperoxia exposure. *J Appl Physiol* 2010;108:1347–1356.
- 21 Almario B, Wu S, Peng J, Alapati D, Chen S, Sosenko IRS: Pentoxifylline and prevention of hyperoxia-induced lung injury in neonatal rats. *Pediatr Res* 2012;71:583–589.
- 22 Amy RW, Bowes D, Burri PH, Haines J, Thurlbeck WM: Postnatal growth of the mouse lung. *J Anat* 1977;124:131–151.
- 23 Lin Y-J, Markham NE, Balasubramaniam V, Tang J-R, Maxey A, Kinsella JP, Abman SH: Inhaled nitric oxide enhances distal lung growth after exposure to hyperoxia in neonatal rats. *Pediatr Res* 2005;58:22–29.
- 24 Jobe AH, Kramer BW, Moss TJ, Newnham J, Ikegami M: Decreased indicators of lung injury with continuous positive expiratory pressure in preterm lambs. *Pediatr Res* 2002;52:387–392.
- 25 Rehan VK, Fong J, Lee R, Sakuri R, Wang Z-M, Dahl MJ, Lane RH, Albertine KH, Torday JS: Mechanism of reduced lung injury by high frequency nasal ventilation in a preterm lamb model of neonatal chronic lung disease. *Pediatr Res* 2011;70:462–466.

Erratum

Figure 1 in the paper by Maschmann J et al. entitled ‘Characterization of human breast milk leukocytes and their potential role in cytomegalovirus transmission to newborns’ [*Neonatology* 2015;107:213–219, DOI: 10.1159/000371753] should read:

Fig. 1. Qualitative parameters of CMV reactivation during lactation of a CMV-transmitting mother (mother A). CMV DNA lactia, pp67 mRNA lactia, and virolactia could be detected in both compartments of breast milk, i.e. milk whey and milk cells (see also fig. 3, 4), respectively. Parameters in boxes were detectable in all sequential weekly samples, while viral DNA lactia and late pp67 mRNA in milk cells were detectable only discontinuously (dotted boxes).