

ALTERED AIRWAY RESPONSIVENESS IN ADULT SHEEP BORN PREMATURELY: EFFECTS OF ALLERGEN EXPOSURE

Kenneth J. Snibson, Robert J. Bischof, and Emmanuel

Koumoundouros □ *Centre for Animal Biotechnology, Department of Veterinary Science, The University of Melbourne, Parkville, Victoria, Australia*

Lachlan S. McMurtrie, Megan Cock, and Richard Harding □ *Department of Physiology, Monash University, Clayton, Victoria, Australia*

□ *The aim of this study was to determine the effects of preterm birth per se on airway function in adult sheep. Preterm birth was induced at ~0.89 of term. At ~1 year of age the authors measured pulmonary resistance (R_L) and airway responsiveness before and after house dust mite (HDM) challenge. Mature preterm sheep tended to have greater baseline R_L than controls ($P = .12$); the smaller preterm sheep showed significantly greater R_L than controls following bronchoconstrictor challenge. Preterm animals tended to have greater baseline total blood leukocyte count ($P = .06$). It was concluded that preterm sheep, especially with low postnatal growth, have greater airway responsiveness to bronchoconstrictor and higher baseline R_L .*

Keywords adult lung function, asthma, hyperresponsiveness, premature birth

Although environmental exposures such as smoking, as well as genetic factors, undoubtedly play important roles in the etiology of impaired lung function and obstructive lung diseases, a significant risk factor that has been identified in a growing number of studies worldwide is low birth weight [1, 2], a major cause of which is preterm birth. With ever-improving obstetric and neonatal management, and the increasing use of reproductive technologies, the number of low-birth-weight, premature infants who are born and survive to become adults is increasing.

Severely preterm birth, especially if it is followed by bronchopulmonary dysplasia (BPD) or chronic lung disease (CLD), is associated with impaired lung function in infants, children [3], and adolescents [4, 5]; in particular

Received 10 February 2006; accepted 18 May 2006.

Address correspondence to Dr. Ken Snibson, Centre for Animal Biotechnology, Department of Veterinary Science, The University of Melbourne, Parkville, Victoria 3010, Australia. E-mail: ksnibson@unimelb.edu.au

small airway function, alveolar formation, and elastic tissue are likely to have been compromised [6]. Preterm birth is also associated with wheeze in infants [7] and children [8], which in turn has been associated with later onset respiratory illnesses such as asthma and COPD [5]. Most of these studies are confounded by the use of mechanical ventilation and/or oxygen supplementation during infancy, each of which has the potential to injure the lungs. It is now recognized that even moderately preterm birth (>34 weeks), with no requirement for ventilatory support, can impair respiratory function in infancy [9] and childhood [8, 10]. Together, these studies suggest that preterm birth *per se* can adversely affect the development of the small airways, terminal respiratory units, and elastic tissue of the lungs. However, there are virtually no data on the underlying mechanisms; little is known about how the lung is affected or what the long-term effects are.

We have recently developed a model of mild-moderate preterm birth in sheep; in this model, premature lambs are born at the limit of viability without the need for ventilatory support. Preterm lambs have altered lung development, at least up to 8 weeks after birth [11, 12]. In particular, airway development is altered, as shown by thicker airway epithelium and increased pulmonary resistance up to 8 weeks after birth. In the present study we have used this model to address the long-term effects of the same degree of preterm birth on airway function in sheep at maturity.

There is now evidence that preterm birth leads to an increased risk of atopy and childhood asthma, and that it is a risk factor in airway hyperresponsiveness [5, 13–15]. Sheep administered airway challenges with a common allergen, house dust mite (HDM), have inflammatory cell responses consistent with the asthmatic condition in humans. In particular, HDM challenge in sheep results in elevated levels of HDM-specific immunoglobulin E (IgE) in the serum and significant recruitment of eosinophils to the bronchoalveolar lavage (BAL) fluid and airway tissues [16]. Repeated challenges with HDM result in remodeling of the airway wall in responsive sheep, similar to that reported for chronic asthmatics [17, 18]. To assess the propensity and the extent that preterm sheep develop asthma-like symptoms, our objectives were to determine the effects of mild-moderate preterm birth, in the absence of postnatal ventilatory support, on (a) pulmonary resistance, (b) airway responsiveness, and (c) allergic airway inflammation before and after HDM challenge in mature animals.

MATERIALS AND METHODS

Animal Groups

All protocols used in this study were approved by the Monash University Animal Welfare Committee. Single-bearing, date-mated Border

Leicester $\times$ Merino ewes were randomly allocated to preterm birth or term birth (control) groups. All lambs were born vaginally. Preterm birth was induced by maternal intravenous (i.v.) administration of 50 mg Epostane (Sanofi-Synthlaw, USA), an inhibitor of the enzyme 3β -hydroxysteroid dehydrogenase, at 131 days after mating; term is approximately 146 days. A low dose of betamethasone (3 mg, intramuscular [i.m]; Celestone Chronodose Schering-Plough, Australia) was administered to both control and preterm pregnant ewes at 131 days of gestation to ensure the survival of lambs in the preterm group [11]; the dose was chosen to ensure neonatal survival without inducing parturition. Control lambs were born at term (145.8 ± 1.3 days; $n = 8$) and preterm lambs were born approximately 2 weeks prematurely (132.2 ± 0.3 days; $n = 7$).

Postnatal Management

After birth, lambs were raised with their mothers; special care including heating, hand-feeding, and physical protection from the mother was necessary during the first few days after birth. Ventilatory support or oxygen supplementation were not required. After weaning, female lambs underwent fallopian tubal ligation before reaching reproductive age to avoid pregnancy. All sheep grew to maturity in large pens with daily exposure to pasture.

Experimental Design

The key elements of the experimental design are outlined in Figure 1. Control ($n = 8$) and preterm sheep ($n = 7$), at 9 to 13 months of age, were sensitized to HDM by subcutaneous injection of HDM in alum [16]. Baseline lung function and airway sensitivity to carbachol were assessed during the sensitization period. To induce pulmonary sensitization to HDM, all sheep were given a primary lung challenge of aerosolised HDM 3 weeks after systemic HDM sensitization. A secondary aerosolized HDM lung challenge was given 1 week after the primary lung challenge. Lung function was recorded for 1 hour directly after the secondary aerosol HDM challenge, and again 24 hours post challenge. BAL cells were sampled from all sheep directly before, and 48 hours after, the secondary HDM challenge.

Measurement of Lung Function

Data on lung function were obtained from all sheep using established techniques [19, 20]. Before each procedure, sheep were mildly sedated with xylazine (Xylazil-20; Troy Laboratories, Australia; ~ 0.05 mg/kg i.m.) and rested in a restraining harness. After topical anaesthesia of the nasal

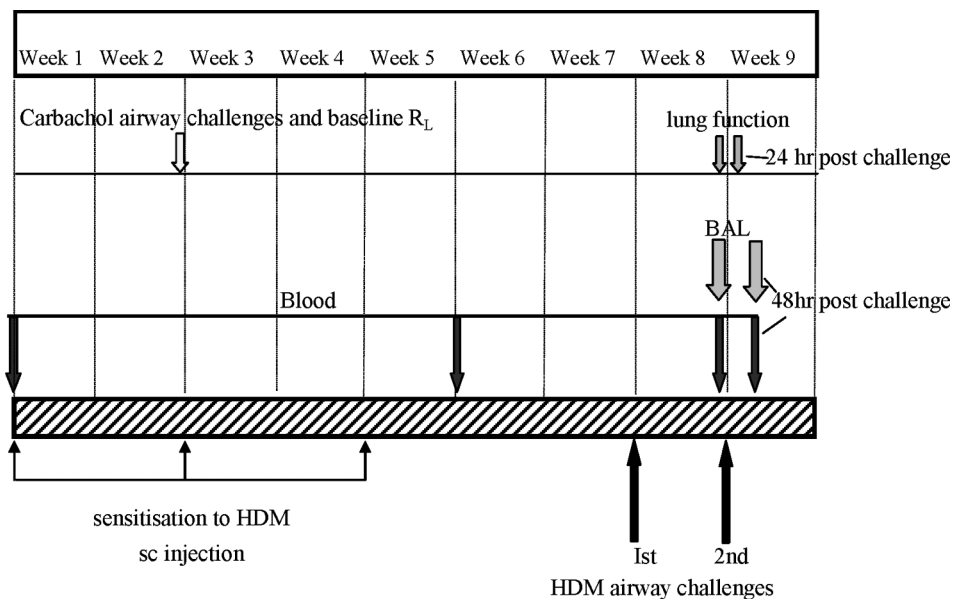


FIGURE 1 Experimental outline. Allergen sensitization started in week 1 and preceded lung function assessments in 9 to 13-month-old preterm and control sheep. Lung function refers to an assessment of pulmonary resistance as outlined in Materials and Methods. HDM, house dust mite; BAL, bronchoalveolar lavage.

passages (2% Lignocaine Gel; Orion, Australia), a balloon catheter was passed through a nostril into the lower oesophagus. Through the other nostril, a cuffed endotracheal tube was inserted under bronchoscopic guidance. A catheter was positioned such that its tip lay 5 cm past the distal end of the endotracheal tube. The esophageal and tracheal catheters were used to measure transpulmonary pressure. Airflow measurements were obtained via a pneumotachograph attached to the proximal end of the endotracheal tube. Calibration and synchronization of the gas transducers and pneumotachograph were performed as described previously [19, 20]. Acquisition and detailed analysis of pulmonary physiological data were performed on customized Labview software developed in house by EK. This software analyzed flow and pressure data from an average of at least 5 breaths; it excluded data from breaths that differ by more than 20% in volume from the previous breath. Baseline R_L was calculated after an equilibration period of 20 minutes following intubation as the mean of 5 to 15 readings, immediately prior to the carbachol challenge test.

All aerosols were generated with a medical nebulizer (Vitalair Rapid-Neb; Allersearch, Vermont, Australia). The nebulizer was connected to a dosimeter system consisting of a solenoid valve and a source of compressed air (20 psi). The output of the nebulizer was directed into a T-piece with

one arm connected to the inspiratory port of a respirator (Intermed Bear 2 Adult Volume Ventilator; Bear Medical Systems, Riverside, CA). Aerosols were delivered at a tidal volume of 500 mL and a rate of 20 breaths per minute for 20 breaths.

Airway Responsiveness

Airway responsiveness to a bronchoconstrictor (carbachol) was assessed in all sheep 2 days before HDM challenge. This was achieved by administering carbachol by aerosol in a range of doubling doses (0.25% to 4% carbachol *w/v*). Each dose of carbachol was aerosolized and delivered in 20 breaths to mechanically ventilated sheep. Airway responsiveness was determined from cumulative concentration-response curves to inhaled carbachol by measuring lung resistance (R_L) immediately after inhalation of aerosol vehicle (phosphate-buffered saline) and after each consecutive administration of increasing concentrations of carbachol. The provocation test was discontinued when the highest carbachol dose had been administered or the pulmonary resistance had increased by over 200% above baseline values. Cumulative concentrations of administered carbachol were expressed in breath units. One breath unit was defined as the dose received from one breath of carbachol aerosolised from 1.0% *w/v* carbachol solution. Airway responsiveness was expressed as the number of breath units of carbachol aerosol needed to increase airway resistance by 200% from baseline.

HDM Sensitization and Airway Challenges

Animals were subcutaneously immunized with 50 μ g house dust mite (HDM) preparation *Dermatophagoides pteronyssinus*; CSL, Parkville, VIC, Australia) mixed 1:1 in aluminium hydroxide ($\text{Al}(\text{OH})_3$) adjuvant. Injections of 1 mL HDM/ $\text{Al}(\text{OH})_3$ were given on three occasions at 2-week intervals at time points outlined in the experimental design (Figure 1) [16]. Levels of HDM-specific IgE (IgE_{HDM}) were determined by enzyme-linked immunosorbent assay (ELISA) of serum samples obtained from animals immediately prior to, and 7 days after, HDM sensitization [16, 21]. Both preterm and control animals showed similarly increased levels of IgE_{HDM} ($P < .05$, preterm; $P < .08$, control) in the postsensitization period. Whole-lung HDM challenges consisted of delivering aerosolized HDM (7 mL of 1 mg/mL HDM) to mechanically ventilated sheep for 15 minutes at 20 breaths per minute.

Leukocyte Analysis in Blood and Bronchoalveolar Lavage Fluid

Peripheral blood samples were taken from the carotid artery prior to and at 48 hours after the secondary HDM aerosol administration. Differential

leukocyte counts were determined by classifying 200 cells as either mononuclear cells, neutrophils, or eosinophils based on their morphology.

Bronchoalveolar lavage (BAL) was performed on each animal immediately prior to, and 48 hours after, the secondary HDM airway challenge. BAL samples were obtained by infusing 10 mL of saline via the bronchoscope biopsy port into the left caudal lobe of the lung and retrieving approximately 4 to 6 mL BAL fluid/cells. BAL cells were isolated by centrifugation and differentially stained with Diff Quik [18]. For each animal differential leukocyte counts in BAL samples were determined by classifying 200 cells as either macrophages, neutrophils, eosinophils, or lymphocytes, based on morphological appearance.

Statistical Analysis

Animal growth was analyzed using a *t* statistic to compare linear regression slopes. Analysis of data on body weights, postnatal ages, R_L 200%, and baseline R_L between control and preterm groups was undertaken using an independent-sample *t* test. Data for dose-response curves for carbachol were analyzed using a repeated-measure analysis of variance (ANOVA), with time (repeated) and treatment group as factors [22, 23]. Least significant difference (LSD) or Games-Howell post hoc tests were employed, depending on the significance of Levene's statistic. Correlations between body weight, postnatal age, and responsiveness to carbachol were undertaken using Pearson's correlation coefficient.

IgE_{HDM} levels and BAL and blood leukocyte counts and differential counts were all analyzed using a repeated-measure ANOVA, with time (repeated) and treatment group as factors. Where a significant effect of age or group was observed, either paired- or independent *t* tests were used for post hoc analysis as appropriate. Pulmonary resistance (R_L) and blood gas responses to HDM challenge were analysed using non parametric statistics: the Mann-Whitney *U* test for between-group comparisons, and the Wilcoxon signed ranks test for within treatment group comparisons. Correlations between birthweight, body weight, postnatal age (PNA), IgE_{HDM}, blood gas parameters, leukocyte counts, and differential counts and R_L response to HDM airway challenge were undertaken using Spearman's rank correlation coefficient.

RESULTS

Postnatal Growth

Analysis of growth curves revealed that sheep, in both control and preterm groups, could be segregated into distinct subgroups according to their

TABLE 1 Body Weight and Growth Rate Data

Sheep no. (#)	Sex	Birth weight	PNA*	Adult weight*	Growth rate (kg/day) [#]
Control					
43	f	4.58	405	59	0.21
44	m	4.55	405	55.8	0.17
42	f	5.05	405	55.8	0.22
47	f	3.25	363	47.8	0.27
50	m	4.7	363	45	0.16
70	f	2.85	293	31.9 ^{&}	0.16
71	f	2.45	293	32.8 ^{&}	0.13
78	f	4.05	262	34.7 ^{&}	0.16
Mean		3.94	348.63	45.35	0.19
SD		0.96	58.11	11.10	0.05
Preterm					
45	f	2.55	354	40	0.29
48	f	2.7	383	51.6	0.33
49	f	3.4	354	59	0.25
69	f	1.6	327	30.9 ^{&}	0.14
74	m	2.75	323	33.75 ^{&}	0.13
75	f	3.08	323	41.25	0.14
76	f	3.12	284	30.9 ^{&}	0.19
Mean		2.74	335.43	41.06	0.21
SD		0.58	31.56	10.75	0.08

PNA: postnatal age.

*Recorded at the time of first HDM (house dust mite) airway challenge.

[#] Postnatal growth rate during postnatal days 0 to 120.

[&] < 35 kg subgroup ($P < .025$).

weight at the time of study (control < 35 kg $n = 3$; > 35 kg $n = 5$; preterm < 35 kg $n = 4$; > 35 kg $n = 3$), on the basis of their significantly different rates of growth (preterm $P < .025$; control $P < .025$) (Table 1). The lighter animals had been separated from other sheep at an early age and had ample access to food, yet still gained weight at a reduced rate compared to their heavier peers. The slower growth rates of the lighter animals were not dependent on birth weight as these animals did not have significantly lower birth weights than the heavier animals (Table 1).

Baseline Pulmonary Resistance

There was a trend towards increased baseline R_L in the preterm animals (mean group values: control 1.8 ± 0.8 , preterm 2.6 ± 0.8 cm $H_2O \cdot s \cdot L^{-1}$; $P = .12$). There also appeared to be an effect of animal weight on baseline R_L such that lighter animals (< 35 kg) had higher baseline R_L compared to heavier (> 35 kg) animals within their treatment group, although these differences were not significant (Figure 2). Postnatal age did not correlate with baseline R_L .

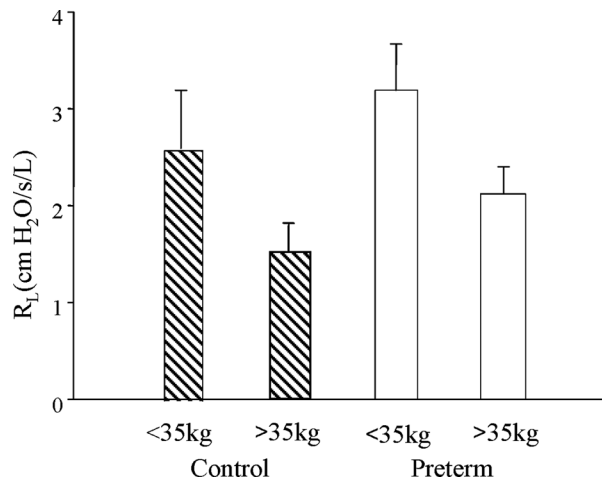


FIGURE 2 Mean baseline pulmonary resistance in 2 weight subsets of preterm and control sheep. Results are mean values for each group with standard deviation error bars. The calculation of lung resistance (R_L) is defined in Materials and Methods. The adult weight groups are based on data presented in Table 1. Control term sheep $n = 8$, preterm sheep $n = 7$.

Airway Responsiveness to Carbachol

Airway responsiveness, as determined by the dose of carbachol required to increase R_L to 200% of the postvehicle value (R_L 200%), was significantly higher in the lower weight preterm animals compared to higher weight preterm animals (Figure 3). The mean number of breath units of carbachol to reach R_L 200% was $18 (\pm 7)$ (highly responsive to carbachol) in lower weight preterm sheep compared to $133 (\pm 10)$ (less responsive to carbachol) for the heavier preterm animals ($P < .004$). In contrast, there was no significant difference in airway responsiveness between the 2 body-weight subsets in the controls 91 ± 14 and 79 ± 41 breath units carbachol to reach R_L 200% for small control, and larger control sheep, respectively; $P = .2$ (Figure 3).

Allergen-Specific Airway Responses

Pulmonary resistance data at 1 hour after the HDM airway challenge, and at 24 hours post challenge, were obtained in 6 control and 4 preterm sheep. Airway challenge with HDM caused a significant increase in R_L in both groups ($P < .05$) compared to the pre-HDM level (Figure 4). There was no significant difference in the R_L reached by each treatment group post HDM challenge within the 1st hour. Both control ($P = .17$) and preterm ($P = .11$) animals displayed a trend towards increased R_L compared

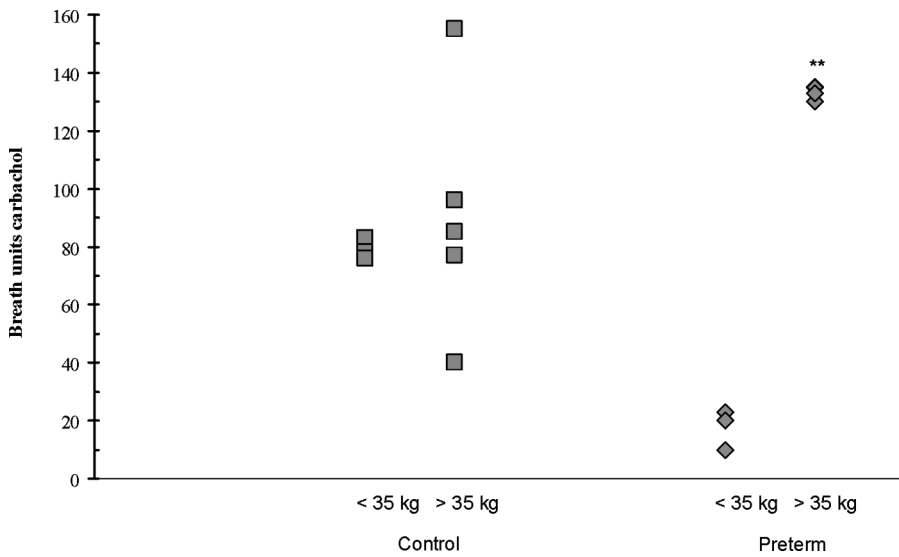


FIGURE 3 Bronchial responsiveness in two weight subsets of preterm and control (term) sheep. Each data point represents the number of breath units (BU) of carbachol necessary to increase pulmonary resistance by 200%. One BU represents one breath of 1% (*w/v*) carbachol. The body weight subsets are indicated on the *x* axis. ** $P < .004$, < 35 kg versus > 35 kg.

to pre-HDM at 24 hours post challenge. There was also a trend towards higher R_L in preterm animals than in controls at 24 hours (control 2.43 ± 0.27 , preterm 3.74 ± 0.81 ; $P = .19$).

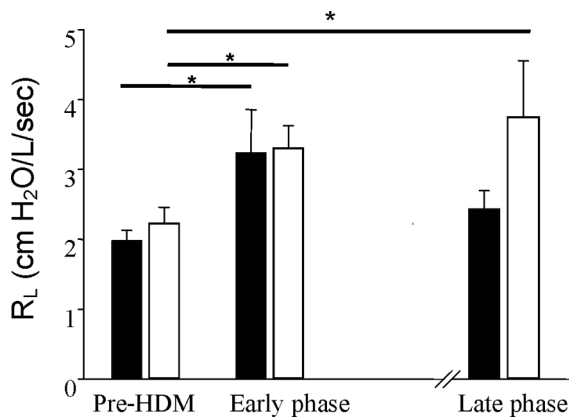


FIGURE 4 Pulmonary resistance responses to HDM airway challenge in term and preterm animals: The early phase response represents the maximum resistance reached during the 1st hour post HDM challenge. The late phase resistance was assessed 24 hours after HDM challenge. * $P < .05$. For pre-HDM 'control' $n = 7$, 'preterm' $n = 6$. For early phase response 'control' $n = 6$, 'preterm' $n = 6$. For late phase responses 'control' $n = 7$, 'preterm' $n = 4$. Filled bars represent control term sheep. Open bars represent preterm sheep. HDM, house dust mite; R_L , pulmonary resistance.

BAL Leukocytes

Before HDM challenge via the airways, there was no significant difference between the percentage of neutrophils and eosinophils in control and preterm animals. HDM airway challenge resulted in a significant increase in the percentage of eosinophils ($P < .01$) and neutrophils ($P < .001$) in BAL fluid at 48 hours postchallenge in both preterm and control groups. This was accompanied by a significant fall in the percentage of macrophages ($P < .001$) and no change in lymphocytes at 48 hours post

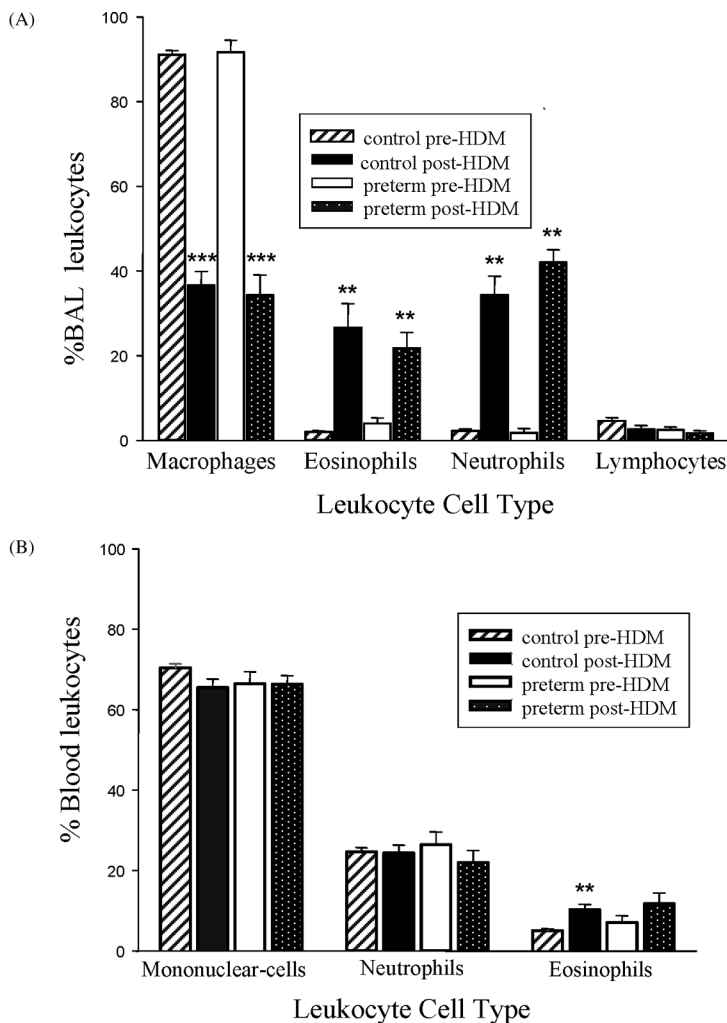


FIGURE 5 Mean values for differential leukocyte counts in (A) bronchoalveolar lavage (BAL) and (B) blood samples pre- and 48 hours post HDM airway challenge. The data are expressed as percentages ($\pm$ SD) of total leukocytes. *** $P < .001$; ** $P < .01$. Control term sheep $n = 8$; preterm sheep $n = 7$.

challenge (Figure 5A). There was no significant effect of prematurity on the magnitude of the increases in eosinophil and neutrophil percentages after HDM challenge.

Blood Leukocytes

There was a trend ($P = 0.06$) towards greater resting (pre-HDM) leukocyte counts in preterm animals compared to controls (Figure 5B). HDM airway challenge significantly increased ($P < .05$) the total blood leukocyte count in both the control and preterm groups at 48 hours post challenge (data not shown). Prematurity had no significant effect on the magnitude of this response. HDM airway challenge significantly increased the proportion of eosinophils in blood leukocytes in control animals ($P < .01$), yet only caused a trend towards an increase in the preterm animals ($P = .11$) at 48 hours post challenge (Figure 5B).

DISCUSSION

The present study was undertaken to determine the effects of preterm birth per se on pulmonary function in young adult sheep under resting conditions and after allergic lung challenges with the important asthma allergen HDM. In a recent study, we found that the same degree of prematurity resulted in an increased airway resistance and thickened airway epithelium in lambs [11, 12]. Our present study suggests that altered airway function persists into adulthood, as young adult prematurely born sheep tended to have greater baseline R_L than controls, especially in those that grew at slower rates after birth. The reasons for this are not clear, but could involve an alteration in lung architecture or airway structure. Morphometric data from our earlier study is consistent with this notion in that we found an increased thickness of the airway epithelium, from the trachea to the alveoli, in prematurely born lambs at 2 and 8 weeks after birth [11]. Similar changes, if persisting into adulthood, could have contributed to the increased R_L we find here. A reduced number of alveolar tethering points is not likely to account for increased resistance in preterm adult sheep because our previous structural data obtained from 2- and 8-week-old preterm lambs shows similar numbers of alveolar attachments in small airways (unpublished data).

A number of clinical studies have related preterm birth to an increased risk of atopy and childhood/teenage asthma [5, 13–15]. However, it is not always apparent from these clinical studies whether this relates to the preterm condition per se or is a consequence of neonatal oxygen and/or ventilatory support, with subsequent lung injury. It is clear from our results that mild preterm birth, in the absence of postnatal ventilatory support, has

some long-lasting effects on pulmonary function in adult sheep challenged with aerosols of allergen. An important finding in this study is that preterm sheep take longer to recover (i.e., return to baseline resistance) after allergen challenge. Compared to control animals, preterm sheep exhibited significantly higher airway resistance 24 hours after aerosol exposure to HDM. This finding suggests that airways of adult sheep born prematurely may be less able to resolve airflow limitation after allergen exposure. It is interesting to note that, although there are differences in late phase responses between groups, the recruitment of inflammatory cells to the BAL and blood in response to HDM challenge was similar in magnitude in both term (control) and preterm sheep. This suggests that it may not be the mere recruitment of inflammatory cells to the lung that is responsible for the late phase response in preterm sheep. We think it more likely that other factors such as altered airway structure or function or perturbed interactions between inflammatory cells, and their mediators, with airway function may be responsible for the slow resolution of allergic responses in preterm sheep. The impaired ability to resolve airflow limitation may be a factor linking prematurity with respiratory symptoms in childhood [8].

The magnitude of the early-phase bronchoconstrictor response, after allergen challenge, was similar in both preterm and term sheep. This indicates that in this experimental setting, mild prematurity does not determine the severity of early-phase bronchoconstrictor responses to allergen. This finding is consistent with cell data from our BAL collections, in that the increased recruitment of immune cells to the airways after HDM challenge was similar in magnitude in preterm and term sheep. The extent of inflammatory cell recruitment to the lungs of HDM-challenged preterm and term sheep is in agreement with our published studies which report marked airway and BAL eosinophilia in adult sheep that have been infused with HDM via the trachea [16, 18]. Early-phase physiological responses and associated immunological responses to allergen challenge appear unaffected by mild preterm birth.

Bronchial hyperresponsiveness has been noted for teenagers born with very low birth weight or young gestational age [5, 14]. The present study clearly shows that the slower growing preterm sheep, as determined by adult weight (<35 kg), exhibit significant bronchial hyperresponsiveness to carbachol challenge. In contrast, term-born and larger preterm sheep do not exhibit increased sensitivity to the bronchoconstrictor carbachol. In this respect it is interesting that airway responsiveness increases with age in adult sheep (our unpublished data). The smaller preterm animals were significantly younger (311 ± 23 days) than their heavier counterparts (353 ± 24 days) at the time of measuring lung mechanics. Because airway responsiveness increases with age in sheep, the younger, low-growth-rate preterm sheep have surprisingly high airway hyperresponsiveness for their age.

The reasons for some of our preterm and control sheep being small as adults is not clear. We note that smaller preterm and control adult sheep were younger when lung function was assessed. The differences in age when sheep were tested was coincidental and an unavoidable consequence of the nature of experiments in which preterm lambs are reared into adulthood. Despite their lower mean age, all smaller sheep show slower growth rates in the first 120 days after birth. It is possible that the lighter preterm and control sheep (<35 kg) were small owing to impaired nutrition, or subclinical infections, in the early postnatal period. Postnatal nutritional restriction and infections have been shown to affect lung structure and function [24–26]. However, the lung function of the low-weight control animals was not different to that of the heavier controls; it is possible that preterm birth combined with restricted nutrition and/or infection after birth could adversely affect lung development. It is of interest in this regard that, owing to poor nutrient intake and utilization, many premature infants grow at slower rates after birth than term infants [27] and that this could contribute to alterations in lung function in these preterm individuals as children and adults.

The mechanisms underlying the increased airway resistance and reactivity of the adult preterm sheep are unclear at present. However, in our previous studies on prematurely born lambs, we found evidence of altered airway development, in that they had a thicker epithelium in large and small airways, and in the alveoli [11, 12]; this thickening was attributed to early exposure to air. Airway reactivity is regulated by communication between the airway epithelium and airway smooth muscle [28], and it is thus possible that this communication was altered in our prematurely born sheep. In this regard it is of interest that respiratory symptoms and illness are greatest in preterm infants and children who had been exposed to long periods of mechanical ventilation and/or oxygen supplementation, both of which have been reported to cause increased thickness of the airway walls [29].

We conclude that mild preterm birth in sheep, especially if followed by slow postnatal growth rates, leads to higher levels of airway responsiveness and a trend for greater baseline R_L . Further studies are needed to determine the basis for these differences.

REFERENCES

- [1] Edwards CA, Osman LM, Godden DJ, Campbell DM, Douglas JG: Relationship between birth weight and adult lung function: controlling for maternal factors. *Thorax*. 2003;58:1061–1065.
- [2] Shaheen S: The beginnings of chronic airflow obstruction. *Br Med Bull*. 1997;53:58–70.
- [3] Chan KN, Wong YC, Silverman M: Relationship between infant lung mechanics and childhood lung function in children of very low birthweight. *Pediatr Pulmonol*. 1990;8:74–81.
- [4] Doyle LW, Cheung MM, Ford GW, Olinsky A, Davis NM, Callanan C: Birth weight <1501 g and respiratory health at age 14. *Arch Dis Child*. 2001;84:40–44.
- [5] Halvorsen T, Skadberg BT, Eide GE, Roksund OD, Carlsen KH, Bakke P: Pulmonary outcome in adolescents of extreme preterm birth: a regional cohort study. *Acta Paediatr*. 2004;93:1294–1300.

- [6] Kennedy JD: Lung function outcome in children of premature birth. *J Paediatr Child Health*. 1999;35:516–521.
- [7] Elder DE, Hagan R, Evans SF, Benninger HR, French NP: Recurrent wheezing in very preterm infants. *Arch Dis Child Fetal Neonatal Ed*. 1996;74:F165–F171.
- [8] Rona RJ, Gulliford MC, Chinn S: Effects of prematurity and intrauterine growth on respiratory health and lung function in childhood. *BMJ*. 1993;306:817–820.
- [9] Hjalmarson O, Sandberg K: Abnormal lung function in healthy preterm infants. *Am J Respir Crit Care Med*. 2002;165:83–87.
- [10] Mansell AL, Driscoll JM, James LS: Pulmonary follow-up of moderately low birth weight infants with and without respiratory distress syndrome. *J Pediatr*. 1987;110:111–115.
- [11] Cock M, Hanna M, Sozo F, Wallace M, Yawno T, Suzuki K, Maritz G, Hooper S, Harding R: Pulmonary function and structure following mild preterm birth in lambs. *Pediatr Pulmonol*. 2005;40:336–348.
- [12] Sozo F, Wallace M, Hanna M, Flecknoe S, Cock M, Maritz G, Harding R, Hooper S: Alveolar epithelial cell differentiation and surfactant protein mRNA following mild preterm birth. *Pediatric Res*. 2006;59:151–156.
- [13] Lucas A, Brooke OG, Cole TJ, Morley R, Bamford MF: Food and drug reactions, wheezing, and eczema in preterm infants. *Arch Dis Child*. 1990;65:411–415.
- [14] Mai XM, Gaddlin PO, Nilsson L, Finnstrom O, Bjorksten B, Jenmalm MC, Leijon I: Asthma, lung function and allergy in 12-year-old children with very low birth weight: a prospective study. *Pediatr Allergy Immunol*. 2003;14:184–192.
- [15] Miller JE: Predictors of asthma in young children: does reporting source affect our conclusions? *Am J Epidemiol*. 2001;154:245–250.
- [16] Bischof R, Snibson KJ, Shaw R, Meeusen EN: Induction of allergic inflammation in the lungs of sensitized sheep after local challenge with house dust mite. *Clin Exp Allergy*. 2003;33:367–375.
- [17] Kariyawasam HH, Robinson DS: Airway remodelling in asthma: models and supermodels? *Clin Exp Allergy*. 2005;35:117–121.
- [18] Snibson KJ, Bischof RJ, Slocombe RF, Meeusen EN: Airway remodeling and inflammation in sheep lungs after chronic airway challenge with house dust mite. *Clin Exp Allergy*. 2005;35:146–152.
- [19] Abraham WM, Delehunt JC, Yerger L, Marchette B: Characterization of a late phase pulmonary response after antigen challenge in allergic sheep. *Am Rev Respir Dis*. 1983;128:839–844.
- [20] Rice AJ, Reynolds PN, Reynolds AM, Holmes MD, Scicchitano R: Tachykinin-induced bronchoconstriction in sheep is NK-1 receptor mediated and exhibits tachyphylaxis. *Respirology*. 2001;6:113–123.
- [21] Shaw RJ, McNeill MM, Gatehouse TK, Douch PG: Quantification of total sheep IgE concentration using anti-ovine IgE monoclonal antibodies in an enzyme immunoassay. *Vet Immunol Immunopathol*. 1997;57:253–265.
- [22] Briggs A, Clark T, Wolstenholme J, Clarke P: Missing, presumed at random: cost-analysis of incomplete data. *Health Econ*. 2003;12:377–392.
- [23] Engels JM, Diehr P: Imputation of missing longitudinal data: a comparison of methods. *J Clin Epidemiol*. 2003;56:968–976.
- [24] Nair RH, Kesavachandran C, Shashidhar S: Spirometric impairments in undernourished children. *Indian J Physiol Pharmacol*. 1999;43:467–473.
- [25] Ong TJ, Mehta A, Ogston S, Mukhopadhyay S: Prediction of lung function in the inadequately nourished. *Arch Dis Child*. 1998;79:18–21.
- [26] Primhak R, Coates FS: Malnutrition and peak expiratory flow rate. *Eur Respir J*. 1988;1:801–803.
- [27] Ehrenkranz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright LL, Katsikiotis V, Tyson JE, Oh W, Shankaran S, Bauer CR, Korones SB, Stoll BJ, Stevenson DK, Papile LA: Longitudinal growth of hospitalized very low birth weight infants. *Pediatrics*. 1999;104:280–289.
- [28] Bertrand C, Tschirhart E: Epithelial factors: modulation of the airway smooth muscle tone. *Fundam Clin Pharmacol*. 1993;7:261–273.
- [29] Shaffer TH, Wolfson MR, Panitch HB: Airway structure, function and development in health and disease. *Paediatr Anaesth*. 2004;14:3–14.