DOI:
10.1002/ppul.24631

Document Version
Peer reviewed version

Lung function and respiratory outcomes in teenage boys and girls born very prematurely

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Statement of Financial support: The research was supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. JLP is an NIHR Senior Investigator.

Key words: Prematurity, respiratory function, sex

Running title: Prematurity, sex and respiratory outcome

ABSTRACT

Objectives: Male sex in prematurely born infants has been associated with worse respiratory outcomes in early childhood.

Working hypothesis: Respiratory outcomes at 11 to 14 years of age in children born very prematurely and routinely exposed to antenatal corticosteroids and postnatal surfactant would differ according to sex.

Study design: Analysis of follow-up data

Patient-subject selection: Three hundred and nineteen children born prior to 29 weeks of gestational age from the United Kingdom Oscillation Study.

Methodology: Spirometry was used to assess forced expiratory flow at 75%, 50% and 25% of expired vital capacity (FEF_{75}, FEF_{50} and FEF_{25}), forced expiratory volume in one second (FEV_1), peak expiratory flow (PEF) and forced vital capacity (FVC). Lung volumes were measured using a helium dilution technique (FRC_{He}) and by plethysmography (FRC_{pleth}). Total lung capacity (TLC) and residual volume (RV) were calculated. Mean lung function measurements were compared using linear mixed models and reported as unadjusted and adjusted for neonatal and age 11-14 years factors. The participants also completed health questionnaires and provided a urine sample for assessment of passive or active smoking.

Results: Three (FEF_{25}, FEF_{25-75}, FEV_1) lung function measures showed significant differences in favour of females after adjustment. The percentage of children with abnormal lung function (below fifth centile for normal) had adjusted differences between 10 and 30 percentage points e.g. for FEF_{25} 15% females compared to 26% males.

Conclusions: Amongst extremely prematurely born school children airway function was significantly worse in males.

INTRODUCTION

Male sex in prematurely born infants has been associated with increased neonatal mortality [1]; one study reporting male infants being 1.6 times (95% CI 1.27 to 2.01) more likely to die than female infants at gestational ages less than 26 weeks [2]. Male infants are also more likely to develop bronchopulmonary dysplasia (BPD) (2.08, 95% CI 1.48 to 2.91) [1]. Amongst 724 infants born at less than 29 weeks of gestation male sex was not only a risk factor for BPD, but also respiratory morbidity in the first year post neonatal unit discharge [3.] Indeed, male infants are more likely to die or develop any major morbidity [4]. We have demonstrated that male sex was a risk factor for death or oxygen dependency at 36weeks post conceptional age, pulmonary haemorrhage, major cranial ultrasound abnormality and respiratory morbidity in infancy after adjustment for neonatal factors in infants born before 29 weeks of gestation [5,6].

Male sex has been associated with lower lung function in healthy preterm infants compared to females [7]. Bentsen et al also reported that male sex was associated with lower lung function at discharge or term equivalent age in infants born prior to 28 weeks of gestational age [8]. Males were also more likely to have worse airways resistance (Raw) at one year of age [9] and be more likely to require bronchodilator therapy at two year follow-up [5]. In early childhood, boys born at less than 32 weeks of gestation had higher rates of hospital admission between three and five years of age than girls; the most common reasons for readmissions were respiratory infections [10]. At eight to eleven years, male sex was a risk factor for asthma development in preterm children [11]. Those data, however, were collected from cohorts not routinely exposed to antenatal corticosteroids or postnatal surfactant. It is, therefore, not clear what the influence of sex on school age lung function might be in such a population. We, thus, have analysed the effect of sex on respiratory outcomes at 11 to 14 years of age in

the United Kingdom Oscillation Study (UKOS) cohort who were routinely exposed to antenatal corticosteroids and postnatal surfactant.

MATERIALS AND METHODS

Lung function results were analysed from children who had been entered into UKOS [12] and followed up at 11 to 14 years [13]. Ethical approval for UKOS was granted by the South Thames Multicentre Research Ethics Committee and the centre-specific local research ethics committees. Follow-up at 11 to 14 years was granted by the South West London National Research Ethics Service Committee.

As previously described [13], lung function testing was carried out as per the American Thoracic Society and the European Respiratory Society guidelines. Spirometry was used to assess forced expiratory flow at 75%, 50% and 25% of expired vital capacity (FEF_{75}, FEF_{50} and FEF_{25}), forced expiratory volume in one second (FEV_1), peak expiratory flow (PEF) and forced vital capacity (FVC). Lung volumes were measured using a helium dilution technique (FRC_{He}) and by body plethysmography (FRC_{pleth}). Total lung capacity (TLC) and residual volume (RV) were also calculated. Results were converted to z-scores accounting for height, sex and ethnicity, as appropriate [14-17]. The participants also completed health questionnaires and provided a urine sample for assessment of passive or active smoking [18]. Some children were unable to attend for lung function assessment, but completed the health questionnaire (see Figure 1). Puberty was assessed by questionnaire and converted to Tanner scores. Children with a Tanner score of two or more for breast or genital development indicated entry to puberty [19,20].

Statistics sample size

These data arise from the follow-up of a randomised trial population which obtained lung function assessments on 248 participants, 127 males and 121 females. With this sample size, and assuming a two-sample t test, a difference in means between boys and girls of 0.41 standard deviations can be detected with power 90%. The actual analysis performed in our study was more complex and so was able to detect even smaller differences since it adjusted for baseline factors and allowed for clustering due to multiple births using a mixed model.

Statistics: analysis

Summary statistics are presented as means and standard deviations (continuous data), or number and percentage (categorical data). The comparisons of neonatal and age 11 to 14 years characteristics for males and females were made using linear or logistic mixed models as appropriate. These models allowed for clustering due to multiple births [21]. Mean lung function measurements were compared using linear mixed models and reported as unadjusted and adjusted for neonatal factors: antenatal steroids, birthweight, oxygen dependency at 36 weeks post-menstrual age, neurological impairment, administration of postnatal steroids (dexamethasone) and age 11-14 years factors: age at assessment, pubertal status (reached puberty or not), passive or active smoke exposure assessed by urinary cotinine, presence of a smoker in the home, reported wheeze, antibiotic use, chest medication and hospitalisation in 12 months prior to age 11-14 follow-up. Box plots of lung function z scores by sex were drawn to summarise the data visually. The differences in mean lung function by sex have been additionally reported as the difference in percentage of participants with abnormal lung function using the distributional approach [22, 23]. The lower fifth centile was used to define 'abnormal' for lung function measures where a higher value indicates better outcome and the

upper 95th centile when a lower value indicates a better outcome. Analyses were done using Stata version 14.

RESULTS

Overall, the results of 319 (161 males/157 females) children aged 11-14 years were included, of whom 248 attended for lung function assessments (Figure 1). Children who participated in follow-up were more likely to have mothers of white ethnicity, and who were non-smokers in pregnancy compared to those not recruited. Participants who were recruited were otherwise similar in characteristics to those not recruited (Tables E1 and E2).

Males were significantly more likely to have had BPD (diagnosed if the infant remained oxygen dependent beyond 36 weeks post-conceptional age), to have received postnatal steroids prior to extubation and to have had neurological impairment. At follow-up, males were less likely to have reached puberty and were more likely to be active smokers. There were no other significant differences between the two groups (Table 1).

Females had superior mean airway function when compared to males, which remained significant after adjustment for neonatal and follow-up factors (Table 2, Figure 2). When these differences were expressed as the percentage of children with abnormal lung function, the differences were 26% males versus 15% females (FEF_{25}), 54% versus 34% (FEF_{25-75}) and 23% versus 15% (FEV_1) (Table 3). Sensitivity analyses were conducted to adjust for the two additional factors, mother's ethnicity and mother's smoking in pregnancy as these differed between those recruited and not recruited for follow-up (Tables E1, E2). These new analyses gave estimated differences in mean lung function by sex that were slightly bigger than those in the primary analysis shown in Table 2 (Table E3).

Males tended to have had more wheeze and used chest medication than females, but these differences were not significant (Table 4). Females had a significantly higher reported use of antibiotics (18% versus 9.7%) (Table 4).

DISCUSSION

We have demonstrated that male sex in very prematurely born children was associated with significantly poorer airway function at 11 to 14 years. Those significant differences were not explained by neonatal or adolescent factors. The size of difference expressed as the percentage with abnormal lung function was over 10 percentage points. There was also a tendency for the males to have more wheeze and be more likely to require chest medications, but those differences did not reach statistical significance.

Our results have biological plausibility given the results from animal and fetal studies suggesting that male sex ay adversely affect lung development [24-31]. Males have been shown to be more susceptible to neonatal hyperoxic lung injury as demonstrated in a mouse model of hyperoxia, in which the males had a lower radial alveolar count and, therefore, a greater arrest in lung development [24]. Those adverse respiratory outcomes in males may be explained by sex differences in fetal lung development. Androgen receptors have been demonstrated throughout the fetal lung parenchyma [25]. In one study, embryonic mouse lungs were cultured with dihydrotestosterone or no additives in early gestation and then examined using light microscopy at 0, 24. 48 and 72 hours. At each time point, the lungs cultured with dihydrotestosterone showed increased numbers of lung terminal buds compared to those cultured without dihydrotestosterone ($p<0.001$) [26]. Androgens, however, delay late gestation lung development if the androgen exposure begins in early gestation [26]. Furthermore, Mullerian inhibiting substance from Sertoli cells in male fetal testes was shown to inhibit surfactant production [27, 28] and airway genesis [29]. In prematurely delivered lambs, males had lower lung compliance with altered surfactant phospholipid composition and function [30]. In addition, analysis of amniotic samples taken from 164 pregnant women with no maternal or

fetal abnormalities demonstrated that the lecithin to sphingomyelin ratios reached phospholipid maturity at 33.7 weeks in female fetuses compared to 35.1 weeks in male fetuses [31]. A further explanation for our results are that the males were more likely to be exposed to postnatal steroids which we have shown has a detrimental long term effect on lung function [32].

The EPICURE follow up study showed that lung function did not differ significantly between boys and girls at 10 to 12 years of age [33]. The majority of children included, however, had not been routinely exposed to antenatal steroids or postnatal surfactant. A study of 8 to 9 year old children suggested that boys born between 32 to 36 weeks gestational age had similar a FEV_1 to their term born peers, but the females had significantly worse lung function [34]. When the cohort, however, reached 16 years of age, both groups had deficiencies in FEV_1 compared to their term born peers. At 19 years of age, females compared to males born before 32 weeks of gestation and with a birth weight below 1.5kg had a higher incidence of wheeze (OR 2.0, 95% CI1.2–3.2, $p<0.05$) and shortness of breath during exercise (OR 3.8, 2.0–7.3, $p<0.05$) [35]. The authors postulated that the better outcomes in the males may be explained by dysynaptic lung growth, that is, the independent growth of airways in comparison with the lung parenchyma and air spaces [35]. In girls, growth of airways is proportional to growth of the lung parenchyma, whereas in boys growth of the airways lags behind that of the lung parenchyma, causing a discrepancy between airway and lung size [36]. Different pubertal patterns of thoracic growth between the sexes resulted in an approximately 25% higher lung function in males than in females at the end of puberty [35]. Hence, in the general population both the incidence and prevalence of wheeze and asthma is higher in males than in females until the age of 16 [37], whereas in adulthood, asthma occurs more

frequently in women [37]. Vrijlandt et al postulated a similar process takes place in the prematurely born population [35]. It is then not predictable whether the lung function differences by sex in our population will persist beyond puberty and thus our cohort require further follow up.

Amongst 5,188 boys and 4,902 girls at 10 to 18 years of age, a dose response relationship between smoking and lower levels of FEV_1/FVC and FEF_{25-75} has been reported. Each pack per day of smoking was associated with a 3.2% reduction in FEF_{25-75} for girls (p=0.01) and a 3.5% reduction in FEF_{25-75} for boys (p=0.007). Adolescent girls were more vulnerable than boys to the effects of smoking on the growth of lung function. Girls who did not smoke reached a plateau of lung function at 17 to 18 years, but girls of the same age who smoked experienced a decline in FEV_1 and FEF_{25-75} [38], smoking, however, was only assessed by self-report. In this study, a greater proportion of the males than the females were active smokers as assessed by self-report and urinary cotinine levels. After adjusting for this difference, the males still had inferior lung function.

Our study has strengths and some limitations. Our study is a large cohort of infants born very prematurely routinely exposed to modern neonatal care including exposure to antenatal steroids and postnatal surfactant. The majority of our study population were white and ethnicity is known to influence outcomes in prematurely born infants. Amongst those born before 26 weeks of gestation, being born to a black mother was protective regarding of retinopathy of prematurity [1]. In addition, amongst infants born prior to 29 weeks of gestation, black infants had a lower risk of BPD but an increased risk of first year respiratory morbidity [39]. In this study, however, there was no significant difference according to gender in ethnicity. Whilst this study is a secondary analysis of data obtained as part of the follow up of the

UKOS trial, the mode of ventilation did not differ by gender, nevertheless we adjusted our results for mode of ventilation. As expected more of the males had had BPD and other poor neonatal outcomes, but when the results were adjusted for this, the lung function results remained significantly different in favour of the females.

In summary, we have demonstrated that, at 11 to 14 years, the airway function of male children born prematurely was significantly poorer than females born at a similar gestational age. Whether this difference remains after puberty merits investigation. Importantly, our results emphasise gender should be taken into account when assessing the effect of disease or interventions in this vulnerable population.

ACKNOWLEDGEMENTS

Financial disclosure: The authors have no financial relationships to this article to disclose.

Conflict of interest: The authors have no competing interests to declare.

Author contributions: AG, CH, JP, SC and NM designed the study. SZ and AL undertook the lung function assessments. CH, AB, AG and JP analysed the data. All authors were involved in the production of the manuscript and approved the final version.

REFERENCES

1. Costeloe KL, Hennessy EM, Gibson AT, Marlow N, Wilkinson AR. The EPICure study: outcomes to discharge from hospital for infants born at the threshold of viability. Pediatrics 2000;106:659-671.
2. Costeloe KL, Hennessy EM, Haider S, Stacey F, Marlow N, Draper ES. Short term outcomes after extreme preterm birth in England: comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). BMJ 2012;345:e7976.
3. Keller RL, Feng R, DeMauro SB, Ferkol T, Hardie W, Rogers EE, Stevens TP, Voynow JA, Bellamy SL, Shaw PA, Moore PE, Prematurity and Respiratory Outcomes Program. Bronchopulmonary dysplasia and perinatal characteristics predict one year respiratory outocmes in newborn born at extremely low gestational age: A prospective cohort study. J Pediatr 2017;187:89-97.
4. Ito M, Tamura M, Namba F; Neonatal Research Network of Japan. Role of sex in morbidity and mortality of very premature neonates. Pediatr Int 2017;59:898-905.
5. Peacock JL, Marston L, Marlow N, Calvert SA, Greenough A. Neonatal and infant outcome in boys and girls born very prematurely. Pediatr Res 2012;71:305-310.
6. Greenough A, Limb E, Marston L, Marlow N, Calvert S, Peacock J. Risk factors for respiratory morbidity in infancy after very premature birth. Arch Dis Child Fetal Neonatal Ed 2005;90:F320-F323.
7. Stocks J, Henschen M, Hoo AF, Costeloe K, Dezateux C. Influence of ethnicity and gender on airway function in preterm infants. Am J Respir Crit Care Med 1997;156:1855-1862.

8. Bentsen MH, Markestad T, Oymar K, Halvorsen T. Lung function at term in extremely preterm born infants: a regional prospective cohort study. BMJ Open 2017;7:e016868.

9. Thomas MR, Marston L, Rafferty GF, Calvert S, Marlow N, Peacock JL, Greenough A. Respiratory function of very prematurely born infants at follow up: influence of sex. Arch Dis Child Fetal Neonatal Ed 2006;91:F197-F201.

10. Elisabeth R, Elke G, Vera N, Maria G, Michaela H, Ursula KK. Readmission of preterm infants less than 32 weeks gestation into early childhood: Does gender difference still play a role? Glob Pediatr Health 2014;1:2333794X14549621.

11. Grischkan J, Storfer-Isser A, Rosen CL, Larkin EK, Kirchner HL, South A, Wilson-Costello DC, Martin RJ, Redline S. Variation in childhood asthma among former preterm infants. J Pediatr 2004;144:321-326.

12. Johnson AH, Peacock JL, Greenough A, Marlow N, Limb ES, Marston L, Calvert SA; United Kingdom Oscillation Study Group. High-frequency oscillatory ventilation for the prevention of chronic lung disease of prematurity. N Engl J Med 2002;347:633-642.

13. Zivanovic S, Peacock J, Alcazar-Paris M, Lo JW, Lunt A, Marlow N, Calvert S, Greenough A. Late outcomes of a randomized trial of high-frequency oscillation in neonates. N Engl J Med 2014;370:1121-1130.

14. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Harkinson JL, Ip MS, Zheng J, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012;40:1324-1343.

15. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, Casaburi R, Crapo R, Enright P, van der Grinten CP, et al. Standardisation of the measurement of lung volumes. Eur Respir J 2005;26:511-522.

16. Rosenthal M, Cramer D, Bain SH, et al. Lung function in white children aged 4 to 19 years: II--Single breath analysis and plethysmography. Thorax 1993;48:803-8.

17. Rosenthal M, Bain SH, Cramer D, Helms P, Denison D, Bush A, Warner JO. Lung function in white children aged 4 to 19 years: I--Spirometry. Thorax 1993;48:794-802.

18. Verification SSoB. Biochemical verification of tobacco use and cessation. Nicotine Tob Res 2002;4:149-159.

19. Tanner JM, Whitehouse RH, Takaishi M. Standards from birth to maturity for height, weight, height velocity, and weight velocity: British children, 1965. I. Arch Dis Child 1966;41:454-71.

20. Tanner JM, Whitehouse RH, Takaishi M. Standards from birth to maturity for height, weight, height velocity, and weight velocity: British children, 1965. II. Arch Dis Child 1966;41:613-35.

21 Sauzet Sauzet O, Wright K, Marston L, Brocklehurst P, Peacock JL. Modelling the hierarchical structure in data sets with very small clusters: a simulation study to explore the effect of the proportion of clusters when the outcome is continuous**.** Stat Med 2013;32:1429-1438.

22 Peacock JL, Sauzet O, Ewings SM, Kerry SM. Dichotomizing continuous data while retaining statistical power using a distributional approach. Stat Med 2012;31:3089-3103.

23 Sauzet O, Breckenkamp J, Borde T, Brenne S, David M, Razum O, Peacock JL. A

distributional approach to obtaining adjusted comparisons of proportions of a population at risk. Emerg Themes Epidemiol 2016;13:8.

24 Lingappan K, Jiang W, Wang L, Moorthy B. Sex specific differerences in neonatal hyperoxic lung injury. Am J Physiol Lung Cell Mol Physiol 2016;311:L481-L493.

25 Kimura Y, Suzuki T, Kaneko C, Darnel AD, Akahira J, Ebina M, Nukiwa T, Sasano H. Expression of androgen receptor and 5alpha-reductase types 1 and 2 in early gestation fetal lung: a possible correlation with branching morphogenesis. Clin Sci (Lond) 2003;105:709-713.

26 Levesque BM, Vosatka RJ, Nielsen HC. Dihydrotestosterone stimulates branching morphogenesis, cell proliferation, and programmed cell death in mouse embryonic lung explants. Pediatr Res 2000;47:481-491.

27 Catlin EA, Manganaro TF, Donahoe PK. Mullerian inhibiting substance depresses accumulation in vitro of disaturated phosphatidylcholine in fetal rat lung. Am J Obstet Gynecol 1988;159:1299-1303.

28 Catlin EA, Uitvlugt ND, Donahoe PK, Powell DM, Hayashi M, MacLaughlin DT. Mullerian inhibiting substance blocks epidermal growth factor receptor phosphorylation in fetal rat lung membranes. Metabolism 1991;40:1178-1184.

29 Catlin EA, Tonnu VC, Ebb RG, Pacheco BA, Manganaro TF, Ezzell RM, Donahoe PK, Teixeira J. Mullerian inhibiting substance inhibits branching morphogenesis and induces apoptosis in fetal rat lung. Endocrinol 1997;138:790-796.

30 Ishak N, Hanita T, Sozo F, Maritz G, Harding R, De Matteo R. Sex differences in cardiorespiratory transition and surfactant composition following preterm birth in sheep. Am J Physiol Reg Integ Compar Physiol 2012;303:R778-R779.

31 Fleisher B, Kulovich MV, Hallman M, Gluck L. Lung profile: sex differences in normal pregnancy. Obstet Gynecol 1985;66:327-330.

32 Harris C, Crichton S, Zivanovic S, Lunt A, Calvert S, Marlow N, Peacock JL, Greenough A. Effect of dexamethasone exposure on the neonatal unit on the school age lung function of children born very prematurely. PLoS One 208;13:e0200243.

33 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: the EPICure study. Am J Respir Crit Care Med 2010;182:237-245.

34 Thunqvist P, Gustafsson PM, Schultz ES, Bellander T, Berggren-Brostrom E, Norman M, Wickman M, Melen E, Hallberg J. Lung function at 8 and 16 years after moderate-to-late preterm birth: A prospective cohort study. Pediatrics 2016;137:pii: e20152056.

35 Vrijlandt EJ, Gerritsen J, Boezen HM, Duiverman EJ; the Dutch P-CSG. Gender differences in respiratory symptoms in 19-year-old adults born preterm. Respir Res 2005;6:117.

36 Sennhauser FH, Kuhni CE. Prevalence of respiratory symptoms in Swiss children: is bronchial asthma really more prevalent in boys? Pediatr Pulmonol 1995;19:161-166.

37 Yunginger JW, Reed CE, O'Connell EJ, Melton LJ 3rd, O'Fallon WM, Silverstein MD. A community based study of the epidemiology of asthma. Incidence rates 1964-1983. Am Rev Respir Dis 1992;146:888-894.

38 Gold DR, Wang X, Wypij D, Speizer FE, Ware JH, Dockery DW. Effects of cigarette smoking on lung function in adolescent boys and girls. N Engl J Med 1996;335:931-937.

39 Ryan RM, Feng R, Bazacliu C, Ferkol TW, Ren CL, Mariani TJ, Poindexter BB, Wang F, Moore pe, Prematurity and Respiratory Outcome Programme Investigators. Black race is associated with a lower risk of bronchopulmonary dysplasia. J Pediatr 2019:207:130-135.

FIGURE LEGEND

Figure 1: United Kingdom Oscillation Study CONSORT follow-up diagram

Figure 2: Box plots of all lung function z scores by sex

Table 1: Maternal, neonatal and follow-up characteristics according to sex (N=319 max*)

Factor	Males		Females		Difference (males-females) or odds ratio (OR; males/females) 95% CI
	N	Mean (SD) or % (n)	N	Mean (SD) or % (n)	
Maternal factors					
Mother's age at birth of child	161	29.8 (5.6)	157	29.2 (5.9)	0.16 (-0.40, 0.71)
White ethnicity (mother)	161	89% (143)	157	90% (142)	OR= 0.84 (0.40, 1.74)
Mother smoked in pregnancy	152	21% (32)	140	26% (37)	OR=0.74 (0.42, 1.30)
Antenatal steroids received	160	90% (144)	157	94% (147)	OR=0.61 (0.25, 1.48)
Neonatal/infant factors					
Birthweight (g)	162	915 (210)	157	874 (206)	43.5 (-1.6, 88.7)
Gestational age (wks)	162	26.8 (1.3)	157	26.9 (1.3)	-0.06 (-0.18, 0.05)
Multiple birth	162	24% (39)	157	24% (37)	OR=1.03 (0.56, 1.88)
Surfactant given	162	98% (158)	157	97% (152)	OR=1.30 (0.34, 4.91)
Oxygen dependent at 36 weeks post-menstrual age	162	64% (103)	157	51.0 (80)	OR=1.68 (1.06, 2.67)
Oxygen dependent at 28 days post-menstrual age	162	88% (143)	157	76% (119)	OR=2.40 (1.26, 4.58)
Oxygen dependent at hospital discharge	161	32% (51)	154	13% (20)	OR=3.11 (1.71, 5.63)
Any air leak	162	14% (23)	157	8.3% (13)	OR=1.83 (0.89, 3.76)
Pulmonary haemorrhage	161	7.4% (12)	156	3.9% (6)	OR=2.01 (0.74, 5.49)
Postnatal steroids given prior to extubation	160	34% (55)	154	19% (29)	OR=2.26 (1.35, 3.78)
PDA	161	31% (50)	157	34% (54)	OR=0.86 (0.54, 1.37)
Neurological impairment	162	19% (31)	156	9.0% (14)	OR=2.40 (1.22, 4.72)
NEC	160	8.1% (13)	155	7.7% (12)	OR=1.05 (0.46, 2.39)
Mode of ventilation at birth: HFOV (versus CV)	162	47% (77)	157	53% (83)	OR=0.81 (0.53, 1.23)
Participants at age 11-14 follow-up					
Participant age at lung function assessment	128	12.5 (0.6)	122	12.5 (0.6)	0.0003 (-0.0002, 0.0008)
Participant weight at assessment (kg)	128	44.4 (14.0)	122	44.9 (9.5)	-0.88 (-3.78, 2.02)

Participant height at assessment (cm)	128	152.0 (9.6)	122	152.2 (7.6)	-0.46 (-2.4, 1.5)
Reached puberty at assessment	141	67% (94)	144	87% (125)	OR=0.30 (0.16, 0.58)
Urinary cotinine at assessment:	115		107		
Undetectable exposure		77% (88)		84% (90)	OR=1.0 (reference)
Passive smoke exposure		1.7% (2)		4.7% (5)	OR=0.41 (0.08, 2.17)
Active smoker		22% (25)		11% (12)	OR=2.13 (1.07, 4.86)
Anyone in family smokes	155	30% (47)	146	33% (48)	OR=0.89 (0.54, 1.48)

* Totals do not always add to 319 due to missing data.

Table 2 Lung function at age 11-14 years according to sex (N=248 max[1])

Lung function measure	Total	Males Mean (SD) Max N=127	Females Mean (SD) Max N=121	Difference (95% CI)[2] (males-females)	Adjusted Difference (95% CI)[3] (males-females)	P value[3]
FEF_{75} z-score	248	-1.13 (0.71)	-1.02 (1.04)	-0.11 (-0.32, 0.11)	0.04 (-0.22, 0.30)	0.76
FEF_{50} z-score	248	-1.43 (0.84)	-0.99 (0.92)	**-0.41 (-0.63, -0.19)**	-0.24 (-0.49, 0.00)	0.054
FEF_{25} z-score	248	-1.29 (0.89)	-0.69 (0.89)	**-0.59 (-0.81, -0.37)**	**-0.31 (-0.55, -0.07)**	**0.012**
FEF_{25-75} z-score	231	-1.80 (0.91)	-1.08 (1.11)	**-0.72 (-0.98, -0.46)**	**-0.46 (-0.76, -0.17)**	**0.002**
FEV_1 z-score	248	-1.04 (1.09)	-0.49 (0.97)	**-0.57 (-0.83, -0.32)**	**-0.36 (-0.65, -0.06)**	**0.020**
FVC_1 z-score	248	-0.50 (1.05)	-0.22 (0.88)	**-0.33 (-0.57, -0.09)**	-0.28 (-0.58, 0.02)	0.067
FEV_1/FVC z-score	248	-1.68 (1.85)	-1.21 (1.69)	-0.42 (-0.85, 0.02)	-0.04 (-0.53, 0.44)	0.86
PEF % predicted	247	81.9 (15.3)	84.8 (15.6)	-2.46 (-6.29, 1.36)	0.37 (-4.15, 4.89)	0.87
DLCO z-score	210	-0.85 (0.89)	-1.08 (1.22)	0.24 (-0.05, 0.53)	0.21 (-0.15, 0.57)	0.26
VA (litres)	210	3.54 (0.70)	3.30 (0.50)	**0.21 (0.05, 0.38)**	**0.29 (0.13, 0.45)**	**0.001**
FRC_pleth z-score	218	0.05 (1.45)	-0.24 (1.03)	0.29 (-0.04, 0.63)	0.08 (-0.32, 0.49)	0.68
FRC_he z-score	229	-0.72 (1.18)	-0.64 (0.95)	-0.12 (-0.39, 0.16)	-0.22 (-0.57, 0.12)	0.21
RV z-score	211	0.76 (1.33)	-0.03 (1.07)	**0.76 (0.43, 1.09)**	0.37 (-0.01, 0.75)	0.059
TLC z-score	213	0.43 (1.09)	0.11 (1.02)	**0.30 (0.02, 0.59)**	0.08 (-0.27, 0.44)	0.65
Respiratory resistance % predicted at 5 Hz	237	98.5 (23.9)	93.4 (20.3)	5.35 (-0.27, 10.97)	-0.83 (-7.57, 5.90)	0.81
Respiratory resistance % predicted at 20 Hz	237	94.9 (26.7)	90.7 (18.6)	4.10 (-1.74, 9.94)	2.16 (-4.69, 9.00)	0.54

Notes: 1 totals vary due to missing data (see methods). 2 unadjusted difference allowing for clustering due to presence of multiple births. 3 adjusted difference: adjusted for antenatal steroid use, birthweight, oxygen dependency at 36 wks PMA, postnatal steroids, major

neonatal neurological impairment mode of ventilation at birth, age at follow-up assessment, pubertal status, passive or active exposure to smoking, presence of a smoker in the home, clustering due to presence of multiple births.

Table 3 Percentage with abnormal lung function (<5th centile) according to sex (N=248 max[1])

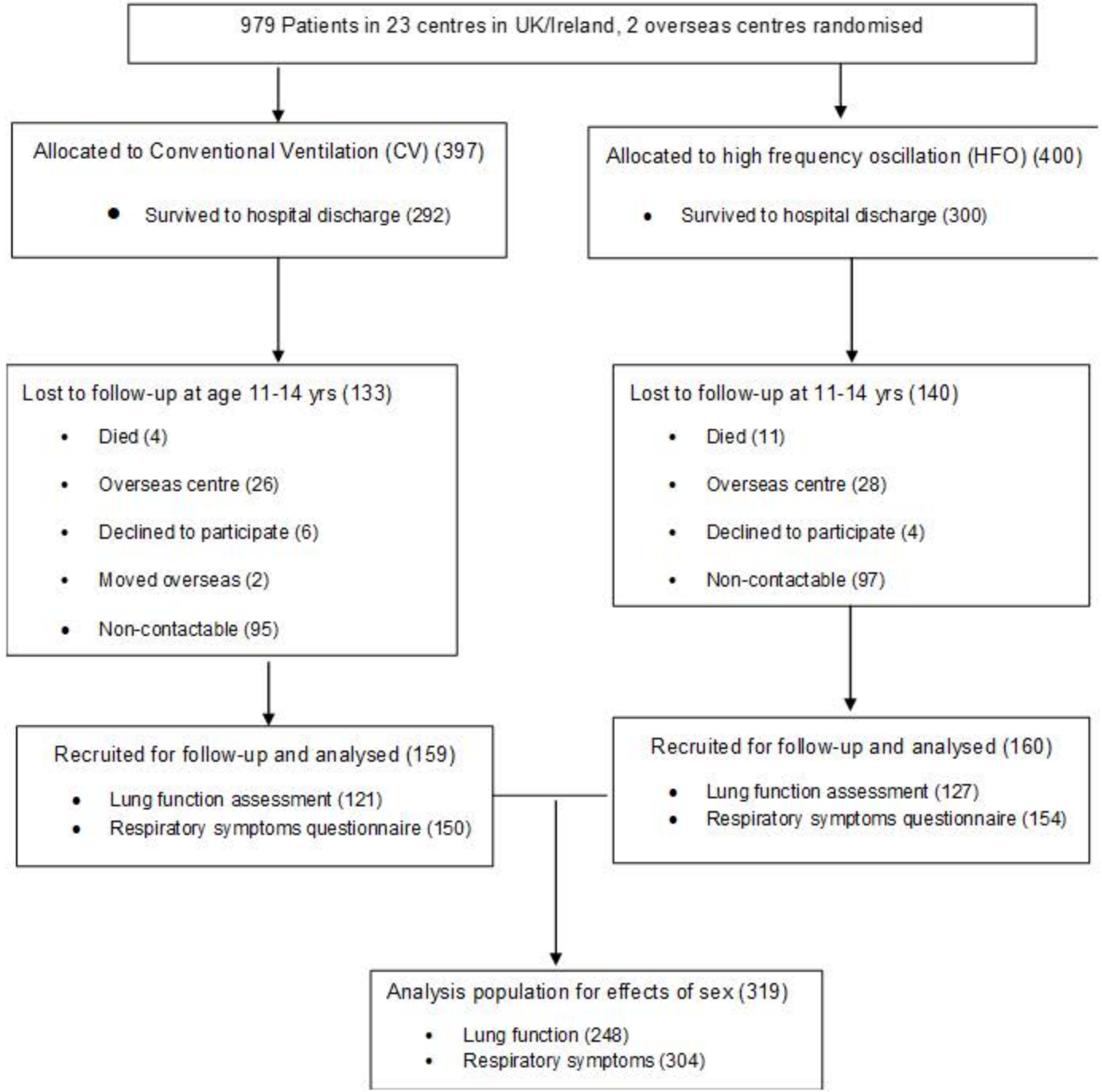
Lung function measure	Total	Males % with abnormal lung function N=127	Females % with abnormal lung function Max N=121	Adjusted difference: percentage points (95% CI)[2] (males-females)	P value[2]
FEF_{75} z-score	248	24.7%	26.3%	-1.6% (-9.3, 6.1)	0.76
FEF_{50} z-score	248	35.9%	24.9%	10.9% (2.7, 19.2)	0.054
FEF_{25} z-score	248	26.3%	14.9%	**11.4% (4.6, 18.1)**	**0.012**
FEF_{25-75} z-score	231	54.0%	34.0%	**20.0% (10.5, 29.5)**	**0.002**
FEV_1 z-score	248	23.3%	13.4%	**9.9% (3.6, 16.3)**	**0.020**
FVC_1 z-score	248	11.7%	6.9%	4.8% (0.8, 8.9)	0.067
FEV_1/FVC z-score	248	46.6%	45.5%	0.1% (-8.4, 10.6)	0.86
PEF % predicted	247	3.0%	3.2%	-0.2% (-1.9, 1.5)	0.87
DLCO z-score	210	21.9%	28.2%	-6.3% (-14.5, 1.9)	0.26
VA (litres)	210	1.0%	0.2%	**0.8% (0.3, 1.3)**	**0.001**
FRC_pleth z-score	218	7.9%	6.9%	1.0% (-2.6, 4.6)	0.68
FRC_he z-score	229	0.9%	1.6%	-0.7% (-1.5, 0.2)	0.21
RV z-score	211	19.4%	11.7%	7.7% (1.6, 13.9)	0.059
TLC z-score	213	23.4%	28.3%	-0.5% (-2.1, 1.1)	0.65
Respiratory resistance % predicted at 5 Hz	237	2.4%	2.7%	0.2% (-1.7, 1.2)	0.81
Respiratory resistance % predicted at 20 Hz	237	2.5%	2.0%	0.5% (-0.8, 1.9)	0.54

Notes: 1 totals vary due to missing data (see methods) 2 adjusted difference in percentages adjusted for antenatal steroid use, birthweight, oxygen dependency at 36 wks PMA, postnatal steroids, major neonatal neurological impairment mode of ventilation at birth, age at follow-up assessment, pubertal status, passive or active exposure to smoking, presence of a smoker in the home, clustering due to presence of multiple births. Uses 'Distributional approach (Peacock 2012, Sauzet 2016 [see refs])

Table 4 Respiratory outcomes in 12 months before follow up (age 11-14 years) according to sex (N=304 max)[1 2]

Outcome	Males % (n/N)	Females % (n/N)	Odds ratio	95% CI	P value
Wheeze	17% (27/156)	12% (18/148)	1.51	0.75, 3.05	0.25
Antibiotic use	9.7% (15/154)	18% (25/141)	0.50	0.26, 0.98	0.044
Chest medicine use	18% (27/153)	12% (18/146)	1.52	0.79, 2.94	0.21
Hospital admission	10% (16/154)	11% (17/148)	0.89	0.42, 1.92	0.77

Notes 1 totals vary due to missing questionnaire data. 2 analyses are adjusted for clustering due to multiple births but further adjustment was not possible due to sample size


979 Patients in 23 centres in UK/Ireland, 2 overseas centres randomised
Allocated to Conventional Ventilation (CV) (397)
Survived to hospital discharge (292)
Allocated to high frequency oscillation (HFO) (400)
Survived to hospital discharge (300)
Lost to follow-up at age 11-14 yrs (133)
Died (4)
Overseas centre (26)
Declined to participate (6)
Moved overseas (2)
Non-contactable (95)
Lost to follow-up at 11-14 yrs (140)
Died (11)
Overseas centre (28)
Declined to participate (4)
Non-contactable (97)
Recruited for follow-up and analysed (159)
Lung function assessment (121)
Respiratory symptoms questionnaire (150)
Recruited for follow-up and analysed (160)
Lung function assessment (127)
Respiratory symptoms questionnaire (154)
Analysis population for effects of sex (319)
Lung function (248)
Respiratory symptoms (304)

Females
Males
FEF75z-score
FEF50z-score
FEF25z-score
FEF25-75z-score
FEV1z-score
FVCz-score
FEV1/FVCz-score
DLCOz-score
FRC_plethz-score
FRC_Hez-score
RVz-score
TLCz-score