

# Prenatal determinants of neonatal lung function in high-risk newborns

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**Background:** Neonatal lung function is suspected to be associated with wheezy disorders, but little is known about risk factors for the early lung function.

**Objectives:** To study prenatal determinants of neonatal lung function.

**Methods:** This is a clinical, prospective birth cohort study of 411 newborns, the Copenhagen Prospective Study on Asthma in Childhood, in a single-center research clinic dedicated solely to this longitudinal birth cohort study. Lung function was determined at 1 month of age by infant spirometry (the raised volume rapid thoraco-abdominal compression technique) and bronchial responsiveness to methacholine by transcutaneous oxygen measurements.

**Risk factor analyses** included anthropometrics; demographics; socioeconomic factors; parental atopic history; previous deliveries; exposures during the third trimester to the mother's smoking, alcohol, and medicines; third trimester pregnancy complications including mother's asthma status; and mode of delivery.

**Results:** Lung function was determined in 404 neonates, age 6 weeks. Neonates with body mass index in the upper quartile had 14% lower baseline forced expiratory volume at 0.5 second, and neonates of mothers smoking during the third trimester had 7% lower baseline forced expiratory volume at 0.5 second. Sex or parental atopic disease did not affect the neonatal lung function and bronchial responsiveness. Maternal intake of paracetamol during the third trimester was associated with doubling of the bronchial responsiveness in the neonates, but the statistical

significance may have been driven by outliers. Bronchial responsiveness exhibited a parabola development with tripling of bronchial responsiveness reaching the nadir at 3 months of age, but this needs replication in a study with repetitive measurements within individuals.

**Conclusion:** High body mass index in newborns and mothers smoking is associated with reduced neonatal lung function. This suggests that the association between body proportion and wheezing disorders may be a result of shared genes or prenatal nutrition. (J Allergy Clin Immunol 2009;123:651-7.)

**Key words:** Neonatal lung function, body mass index, tobacco, paracetamol

Minor alterations in lung structural development during fetal and perinatal life may have marked postnatal consequences leading to critically altered responses to respiratory infections and other insults to the airways. The early lung function tracks into later life,<sup>1-3</sup> and reduced infant lung function has been associated with the development of recurrent wheeze and asthma.<sup>4-9</sup> Therefore, analyses of the neonatal lung function may identify risk factors of importance for the development of lung disease in childhood.

Available risk analyses of neonatal lung function are limited to birth weight, gestational age, and the mother's tobacco smoking. The Copenhagen Prospective Study on Asthma in Childhood (COPSAC) is a clinical study of a birth cohort of 411 infants born to mothers with asthma designed to investigate determinants of childhood asthma prospectively and provides comprehensive, predefined assessments of atopic heredity, anthropometrics, demographics, pregnancy complications, exposures during pregnancy, and circumstances of birth collected prospectively.

Baseline lung function was measured by infant spirometry (raised volume rapid thoraco-abdominal compression technique), and bronchial responsiveness to increasing doses of methacholine was determined by transcutaneous oxygen measurements in the neonates at 1 month of age.<sup>10,11</sup> The objective of this study was to identify prenatal determinants for the neonatal baseline lung function and bronchial responsiveness. The study is reported in accordance with recognized guidelines for observational studies.<sup>12</sup>

## METHODS

### Study design

The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and approved by the Ethics Committee for Copenhagen (KF 01-289/96) and the Danish Data Protection Agency (2008-41-1754). Before enrollment, informed consent was obtained from both parents. Data validity and quality control procedures followed "Good Clinical Practice" guidelines. History was recorded online during visits to the COPSAC clinical

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**Abbreviations used**

BMI: Body mass index  
 COPSAC: Copenhagen Prospective Study on Asthma in Childhood  
 FEV<sub>0.5</sub>: Forced expiratory volume at 0.5 second  
 PD<sub>15</sub>: Provocative dose causing a 15% decrease  
 PtcO<sub>2</sub>: Transcutaneous oxygen pressure

research unit. Objective assessments were double-checked against source data, and the database was subsequently locked and tracked by an audit trail.

The cohort was previously described in detail.<sup>13-15</sup> It includes 411 infants born of mothers with a doctor's diagnosis of asthma, excluding infants with gestational age less than 36 weeks, congenital abnormality, systemic illness, or a history of mechanical ventilation or lower airway infection.

The cohort was included between 1998 and 2001, and the current measurements were performed at the 1-month visit during that period.

All information was collected during personal interviews at the clinical research unit during pregnancy or no later than at the visit 1 month after birth and documented from midwife records. Interviews were conducted by medical doctors with pediatric training employed full-time for this research effort. Structured questions and closed-end response categories were used for online data entry. Prenatal risk factors were primarily analyzed for the third trimester because this is the main period of growth in airway caliber.

Parents from the COPSAC cohort were invited to have the lung function of the infant tested at 1 month of age.

The 2 operators followed written standard operating procedures for all methods, including criteria for accepting or rejecting data.

**Baseline lung function test**

Infant spirometry was obtained by forced flow-volume measurements applying the raised volume rapid thoraco-abdominal compression technique as previously described in detail.<sup>10,11,16-18</sup> The forced expiratory volume at 0.5 second (FEV<sub>0.5</sub>) from spirometry was chosen as the end-point for the baseline lung function on the basis of our previous sensitivity analyses.<sup>10</sup>

The infant was sedated with an oral dose of chloral hydrate of 90 mg/kg and monitored continuously by pulse-oximetry for safety. A nonexpandable outer coat was wrapped around the infant's chest and abdomen with an inflatable balloon inside. The infant's lung volume was raised above the tidal range by inflating air through the pneumotacograph to a pressure of 2 kPa before a forced expiration. A compression force transmitting an additional pressure of 2 kPa was then applied via the squeeze-jacket to the thorax and abdomen, leading to an airway opening pressure of 4 kPa for the forced expirations. The forced expiratory maneuvers were measured 5 times at baseline and 3 times after each challenge step. The median flow measure was used to represent the lung function at each step.

We recently reported the safety and feasibility of the procedure to be good<sup>11</sup> and the mother's experience acceptable as reported in focused interviews.<sup>19</sup>

Application of the method is illustrated by a video at our website at <http://www.copsac.com/standard-operating-procedures/infantspirometry.html>

**Methacholine challenge**

The aerosol was administered with a dosimeter attached to a nebulizer and inhaled from a metal spacer as previously described.<sup>10</sup> After initial inhalation of saline, methacholine chloride was given in quadrupling dose steps from 0.04 to 16.67  $\mu$ mol. The test procedure aimed at a 20% fall in FEV<sub>0.5</sub> or reaching the maximum dose; the median number of administered doses was 4. A 1-mg terbutaline pressurized metered-dose inhaler was administered via spacer at the end of the test.<sup>10</sup>

**Transcutaneous oxygen pressure**

Transcutaneous oxygen pressure (PtcO<sub>2</sub>) after each dose step was used to determine bronchial responsiveness based on previous sensitivity analyses of known indices. PtcO<sub>2</sub> was measured continuously by the TCM3 from Radiometer, Copenhagen, Denmark. The electrode was heated to 44°C and positioned on the flexor side of the lower arm 15 minutes before measurements.

PtcO<sub>2</sub> was determined as mean value during the minute immediately before the spirometry measurement at each dose-step.<sup>10</sup>

**Prenatal variables**

For prenatal variables, see this article's Table E1 in the Online Repository at [www.jacionline.org](http://www.jacionline.org). Demographics included description of sex, ethnicity, parent's age at birth, and mother's previous deliveries. Socioeconomic status was evaluated from household income, mother's educational level, and occupational status. Household income was classified into 3 groups.<sup>13</sup> The highest educational level of the mother was classified into 3 categories: elementary school or college, medium length education, or university education. Occupational status was described in 4 groups—professionals, nonprofessionals, unemployed, and students—based on International Standard Classification of Occupations; International Labor Organization (<http://www.ilo.org>).

Atopic predisposition included parental history of asthma, allergic rhinitis, atopic dermatitis, and urticaria.

Exposures during the third trimester included information on smoking, alcohol, and medications (acetylsalicylic acid, nonsteroidal anti-inflammatory drugs, paracetamol [acetaminophen], systemic steroids, and antibiotics). All exposures were dichotomized.

Pregnancy complications during the third trimester were hemorrhage, preterm contractions, pre-eclampsia, influenza, and pneumonia. All exposures were dichotomized. The mother's asthma status (where applicable) was categorized as better, worse, or unchanged.

The newborn was described by mode of delivery (natural/vacuum/cesarean), Apgar score at 5 minutes, birth weight, and body mass index (BMI) at birth.

Lifespan at examination date was calculated in weeks as the sum of the estimated gestational age and age since birth.

Colonization with *Streptococcus pneumoniae*, *Hemophilus influenzae*, and/or *Moraxella catarrhalis* obtained from hypopharyngeal aspirates at the end of the lung function procedure as previously reported<sup>15</sup> was included in a subanalysis.

**Statistical analyses**

Baseline measures of FEV<sub>0.5</sub> were calculated as the median of repeated measurements. Provocative doses for PtcO<sub>2</sub> were defined at 15% decrease (PD<sub>15</sub>[PtcO<sub>2</sub>]) and were estimated from the dose-response curves for each infant fitted with a nonlinear logistic function<sup>20,21</sup>:

$$f(x) = d / (1 + \exp(b[\log(x) - \log(e)])),$$

where  $f(x)$  is the expected response at methacholine dose  $x$ . The parameters  $d$  and  $b$  are interpreted as response at dose 0 and dose needed to obtain a 50% decrease in response, respectively. The parameter  $b$  is proportional to the slope at  $x = e$ . The estimated dose to obtain a  $K\%$  decrease in response can be calculated by the expression

$$e_K = [eK / (100 - K)]^{1/b}.$$

The association between risk factors and logarithmic lung function outcomes was modeled by linear normal models.

Lifespan at examination date and birth weight were treated as a continuous variable, whereas all other predictor variables were categorical. Groupings were defined from previous knowledge where possible. In all other cases, thresholds were defined empirically by the quantiles of the predictor.

The linearity of the 2 quantitative predictors was tested by comparison with models including second-order and third-order powers of the predictors as well as with the estimated functional form obtained from a general additive model. As a consequence, a quadratic lifespan term was included to improve the fit of the PD<sub>15</sub>(PtcO<sub>2</sub>) model.

Model reduction on the initial multiple regression models including all risk factors was performed by backward elimination with cutoff at a  $P$  value of .05. The model selection procedure was compared with the results obtained from an all subsets regression (where goodness-of-fit was measured by the Akaike information criterion) leading to consistent conclusions. All analyses were performed on complete cases only.

**TABLE I.** Multivariate risk factor analyses of baseline lung function (FEV<sub>0.5</sub>)

| Risk factor                       | Baseline FEV <sub>0.5</sub> |         |          | P value              |
|-----------------------------------|-----------------------------|---------|----------|----------------------|
|                                   | Estimate                    | CI 2.5% | CI 97.5% |                      |
| Age at test (wk)                  | 1.031                       | 1.026   | 1.037    | $<1 \times 10^{-16}$ |
| Weight at birth (kg)              | 1.187                       | 1.118   | 1.259    | $2.9 \times 10^{-8}$ |
| BMI at birth (kg/m <sup>2</sup> ) |                             |         |          | .002                 |
| 7-12                              | Reference                   |         |          |                      |
| 12-13                             | 0.986                       | 0.93    | 1.046    | .646                 |
| 13-14                             | 0.972                       | 0.904   | 1.044    | .433                 |
| 14-17                             | 0.863                       | 0.789   | 0.945    | .002                 |
| Smoking during third trimester    | 0.930                       | 0.878   | 0.985    | .013                 |

To rule out strong effects of possible outliers, the final models were validated by using robust MM-estimators.<sup>22</sup> This estimator can be viewed as a weighted least-squares method that will fit the bulk of the data and is robust in the sense that it weighs down possible outliers at a negligible cost of precision. All data analyses were performed in the freeware statistical program R version 2.7.1.

## RESULTS

Lung function was analyzed in 404 of 411 infants. The median age was 6 weeks at the time of testing. Mean birth length was 52.3 cm and birth weight 3.52 kg. BMI at birth for girls was  $12.84 \pm 1.25$  and for boys was  $12.77 \pm 1.37$ . The covariants analyzed together with univariate analyses of their estimated effect on the baseline FEV<sub>0.5</sub> and PD<sub>15</sub>(PtcO<sub>2</sub>) are presented in Table E1.

### Baseline FEV<sub>0.5</sub>

Baseline FEV<sub>0.5</sub> was obtained in 404 neonates. The multiple regression analysis (Table I) showed that the neonatal lung function was significantly affected by the BMI of the newborn (upper vs lower quartile: 0.863; 0.789-0.945;  $P = .002$ ), birth weight (1.187 per kg; 1.118-1.259;  $P < .0001$ ), lifespan (1.031 per week; 1.026-1.037;  $P < .0001$ ), and the mother's smoking (0.930; 0.878-0.985;  $P = .013$ ). The effect from the mother's smoking was similar in all 3 trimesters (see this article's Fig E1, A, in the Online Repository at [www.jacionline.org](http://www.jacionline.org)). All significant findings were consistent with the estimates from a robust analysis. Notably the baseline lung function was not affected by sex, parents' atopic disease, or bacterial colonization.<sup>15</sup>

Fig 1 together with the online video illustrates the relation between predicted FEV<sub>0.5</sub> and the birth length and weight in a 3-dimensional format. The illustration suggests that for a given birth length, FEV<sub>0.5</sub> increases with birth weight, but with a relative reduction in the heaviest newborns. Also, an interaction between birth weight and birth length is suggested, because the observed effect is less pronounced in the longest of newborns.

The estimated biological variation quantified by the residual SE in the final model for FEV<sub>0.5</sub> was 0.209 on 396 degrees of freedom, and the standard measure of explained variance by the model (adjusted  $R^2$ ) was 0.343.

### Provocative dose, methacholine (PD<sub>15</sub>[PtcO<sub>2</sub>])

PD<sub>15</sub>[PtcO<sub>2</sub>] could be estimated in 363 neonates.

This article's Table E2 in the Online Repository at [www.jacionline.org](http://www.jacionline.org) compares PD<sub>15</sub>(PtcO<sub>2</sub>) obtained by traditional interpolation and by a nonlinear model. This comparison illustrates that the PD<sub>15</sub>(PtcO<sub>2</sub>) estimated by interpolation is systematically lower than PD<sub>15</sub>(PtcO<sub>2</sub>) estimated by nonlinear modeling. This article's

Fig E2 in the Online Repository at [www.jacionline.org](http://www.jacionline.org) exemplifies the strength of the nonlinear model over the linear interpolation. We have therefore used the nonlinear model in the risk-factor analyses.

The multiple regression analysis (Table II) showed that PD<sub>15</sub>(PtcO<sub>2</sub>) fell significantly ( $P < .0001$ ; increased bronchial responsiveness) with lifespan at examination date, with an estimated nadir at a lifespan of 53 weeks (95% CI, 50-55)—that is, approximately 3 months after birth. The estimated drop from birth to nadir was 71% (95% CI, 28% to 88%; Fig 2).

PD<sub>15</sub>(PtcO<sub>2</sub>) increased significantly (reduced bronchial responsiveness) with birth weight (1.513 per kg; 1.077-2.124;  $P = .02$ ). Notably PD<sub>15</sub>[PtcO<sub>2</sub>] was not affected by BMI, sex, parents' atopic disease, or bacterial colonization.<sup>15</sup>

The multiple regression analysis showed that the PD<sub>15</sub>[PtcO<sub>2</sub>] was significantly lower in neonates of mothers using paracetamol in pregnancy (0.551; 0.337-0.900;  $P = .02$ ). However, this borderline significance was not retrieved in the robust analysis eliminating outliers (0.782; 0.517-1.182;  $P = .24$ ).

The estimated biological variation quantified by the residual SE in the final model for PD<sub>15</sub>(PtcO<sub>2</sub>) was 1.686 on 357 degrees of freedom (adjusted  $R^2 = 0.045$ ).

## DISCUSSION

### Summary of principal findings

Baseline FEV<sub>0.5</sub> was 14% lower in neonates with BMI in the upper quartile and 7% lower in neonates whose mothers reported smoking during pregnancy.

Maternal intake of paracetamol during the third trimester was associated with increased bronchial responsiveness (PD<sub>15</sub>[PtcO<sub>2</sub>] reduced) in the neonates, but the statistical significance may have been driven by outliers, as suggested from the lack of significance in the robustness test, taking such outliers into account.

Bronchial responsiveness increased during the first 3 months of life with a tripling of bronchial responsiveness at nadir (provocative dose reduced by 70%).

### Strengths of this study

This is comfortably the largest number of newborns studied for standardized and validated forced spirometry and bronchial responsiveness. Lung function measurements were completed in 404 asymptomatic neonates under standardized conditions in this single-center study.

The cohort was studied within a narrow age range of around 1 month after birth.

Lung function was measured by infant spirometry adapted as the state-of-the-art raised volume rapid thoraco-abdominal

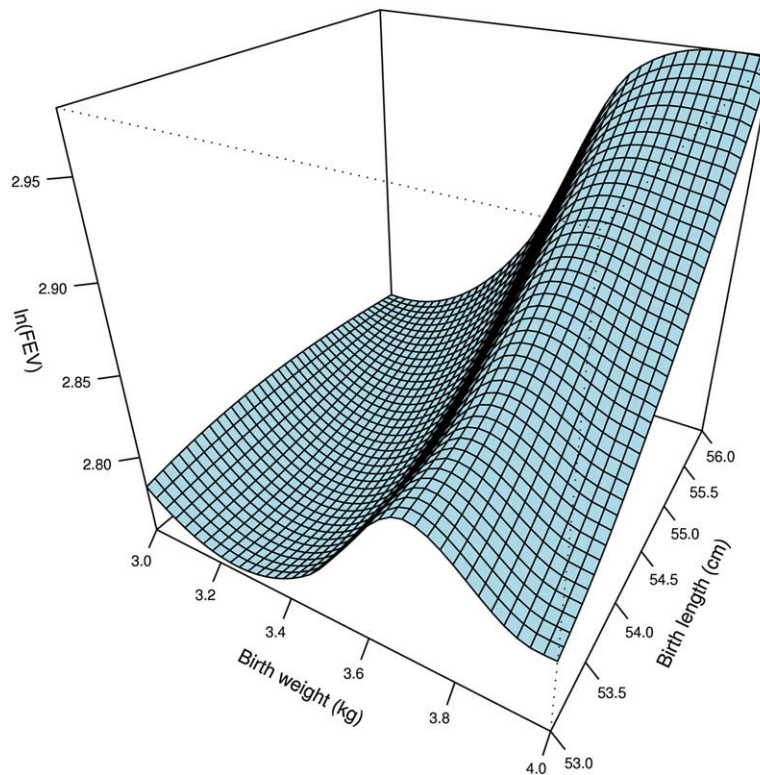


**FIG 1.** BMI and birth weight were both significantly associated with baseline FEV<sub>0.5</sub> in neonates after adjusting for the other significant determinants. The figure illustrates the interaction, estimated using a locally weighted smoother, between birth weight and birth length and using the adjusted *ln* baseline FEV outcome. See online video for different perspectives on these relations. *ln*, Natural logarithm.

**TABLE II.** Multivariate risk factor analyses of bronchial responsiveness (PD<sub>15</sub>PtcO<sub>2</sub>)

| Risk factor                                  | PD <sub>15</sub> PtcO <sub>2</sub> |         |          | P value              |
|----------------------------------------------|------------------------------------|---------|----------|----------------------|
|                                              | Estimate                           | CI 2.5% | CI 97.5% |                      |
| Age at test (wk)                             |                                    |         |          | $2.8 \times 10^{-3}$ |
| Gestational age + current age (wk)           | 0.443                              | 0.2776  | 0.7072   | $6.8 \times 10^{-4}$ |
| [Gestational age + current age] <sup>2</sup> | 1.008                              | 1.003   | 1.012    | $6.2 \times 10^{-4}$ |
| Weight at birth (kg)                         | 1.513                              | 1.077   | 2.124    | .02                  |
| Paracetamol third trimester                  | 0.551                              | 0.337   | 0.900    | .02                  |

compression technique, which provides volume-anchored flow measures.<sup>10</sup> We previously reported measures of PD<sub>15</sub>(PtcO<sub>2</sub>) to be more sensitive for measurements of bronchial responsiveness than flow indices. Therefore, it was our recommendation to use FEV<sub>0.5</sub> for baseline flow measurement and PD<sub>15</sub>(PtcO<sub>2</sub>) for measurements of bronchial responsiveness<sup>10</sup> as in the current risk factors analysis. Bronchial responsiveness was used as a quantitative trait—that is, we did not attempt to dichotomize such measures to define hyperresponsiveness.

We used a nonlinear dose-response model to determine the provocative dose estimates. This approach efficiently uses the data and provides sounder dose estimates than the traditional interpolation of the dose-response relationship, which is more prone to variations. The traditional linear interpolation relies on only 2 measurements, whereas the nonlinear dose-response approach draws on all data, leading to more robust estimates. The dose-response curve is often convex or concave in the interval between 2 adjacent dose points, leading to bias with simple interpolating (Fig E1; Table E2).

The variance of lung function and bronchial responsiveness assessments was comparatively low because of use of dosimetric aerosol delivery and the single-center nature of the study, with 400+ consecutive tests conducted by the same investigators.

The study provides comprehensive prospective assessment of predefined endogenous and environmental factors possibly affecting the development of the neonatal lung function including atopic heredity, anthropometrics, demographics, pregnancy complications, exposures during pregnancy, and circumstances of birth collected prospectively since pregnancy.

### Limitations of this study

The external validity of the conclusions of this study is limited by the cohort selection of newborns, mainly white subjects, all with a gestational age above 35 weeks, with no congenital abnormality, systemic illness, or history of mechanical ventilation or lower airway infection, and born to mothers with asthma. The reason for this selection was primarily the very comprehensive clinical assessments in the cohort including the current lung function

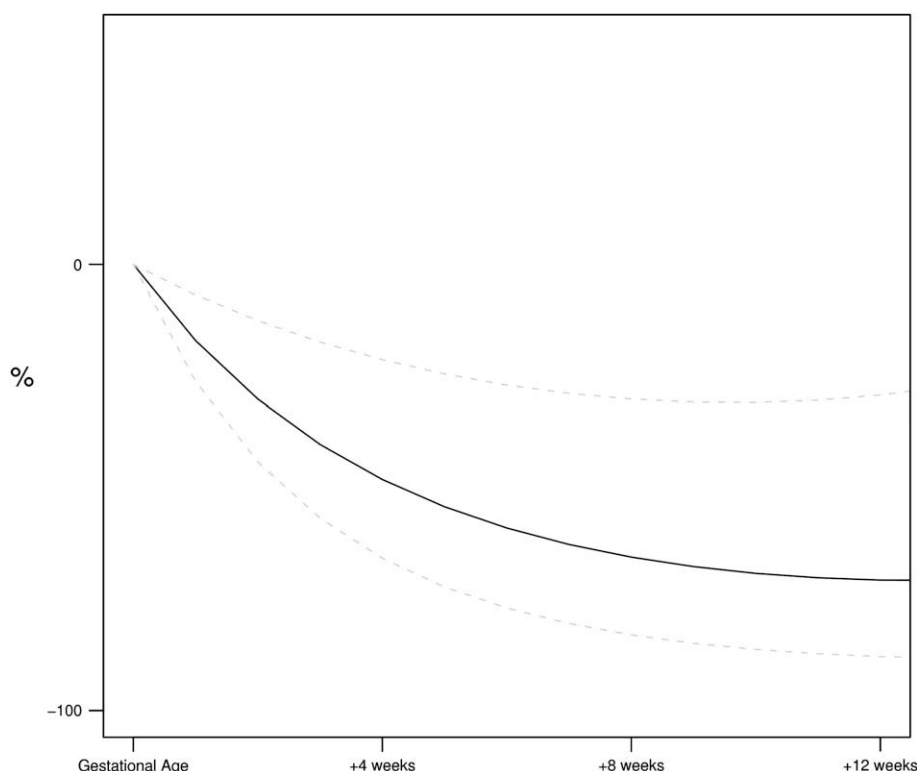


**FIG 2.** Relative change in bronchial responsiveness as a function of age during the first 3 months of life. Dashed lines show the 95% CIs. The median age at examination was 6 weeks (interquartile range, 5–8 weeks) with few subjects studied at the later time points, as reflected in the widening confidence limits.

measurements requiring sedation, which may not be ethically viable in an unselected cohort. However, risk factors and the absolute levels of lung function and bronchial responsiveness may be particular to this selected population and need validation in unselected populations if possible.

Exposures during pregnancy were assessed in personal interviews before or shortly after birth with little risk of recall bias, yet were based only on the mother's history, which is a limitation to the reliability of information such as use of alcohol and tobacco.

The discovery of the tripling of bronchial responsiveness with estimated nadir 3 months after birth should be interpreted with caution because of the cross-sectional nature of the measurements—that is, this is not based on serial measurements. Therefore, the longitudinal changes are inferential and hypothesis-generating only. This observation can be verified only by repeated measurements in the same infants during the first 3 months of life (a challenging study to conduct with current technology).

### Interpretation of the results

It is the novel finding that obese newborns were shown to have lower baseline  $FEV_{0.5}$  than newborns of normal BMI independent of birth weight (Fig 1; online video). Obviously lung function was higher with increasing birth weight and birth length, but for a given length, we found a relative reduction of  $FEV_{0.5}$  in the heaviest neonates. Furthermore, we observed an interaction between birth length and birth weight as illustrated in Fig 1 and the online video, suggesting the effect of BMI was least obvious in the longest newborns.

Notably, the effect was the end result of a multiple regression analysis taking into account conceivable confounders including

gestational age, social status, mother's smoking, breast-feeding, and others. Previous studies have relied mainly on birth weight as the simple estimate of body proportions, and few have included birth length.

Our method standardized the applied compression of the thorax for airway pressure—that is, the thoraco-abdominal compression was adjusted until the target airway pressure was achieved. Therefore,  $FEV_{0.5}$  was not confounded by any mechanical effect of obesity.

Our findings may contribute to an understanding of the recent report on weight-for-length ratio by 6 months of age as a risk factor for recurrent wheeze in young children.<sup>23,24</sup> This observation may in fact be caused by the reduced lung function we have observed in the current study.

The common link between neonatal lung function and obesity may be shared genes or prenatal exposure to specific nutrients or maternal nutrition. Interestingly, both genetic obesity<sup>25</sup> and diet-induced obesity<sup>26</sup> caused innate airway narrowing and airway hyperresponsiveness to methacholine and enhanced inflammation in mouse models. It was speculated that the mechanism works through increased leptin, which has the capacity to induce  $TNF-\alpha$  expression, which again can induce airway hyperresponsiveness.<sup>25,26</sup>

The mother's smoking during pregnancy was associated with 7% lower baseline air flow but did not affect bronchial responsiveness. This finding was similar for smoking ever during pregnancy or smoking in the first, second, or third trimester (Fig E1). The comprehensive confounder adjustment of the current analysis validates previous reports of reduced infant lung function from the mother's smoking.<sup>8,27–30</sup> We suggest that this

loss of lung function by approximately 1 month of age is mainly a result of an effect from the mother's smoking during pregnancy, though some effect from environmental tobacco exposure during this first month of life may have contributed. Starting life with a 7% lower lung function is a significant handicap imposed by a factor that could be avoided and emphasizes the need to improve health education and legal regulations to protect the fetus.

The mother's use of paracetamol was associated with a doubling of bronchial responsiveness in the newborn, albeit with a wide CI and failing statistical significance when outliers were removed in a robustness test. This borderline significance could be found because paracetamol was a spurious finding or because of insufficient study power. Indeed, there is mounting evidence that paracetamol use in pregnancy<sup>31-33</sup> and current paracetamol use<sup>34-40</sup> increase the risk of asthma. The mechanism of such an association is unknown, but it has been speculated that the effect may act through reduction of glutathione, weakening the ability to mitigate oxidative stress.<sup>41</sup>

It is noteworthy that neonatal lung function was not affected by the mother's eczema, urticaria, or allergic rhinitis or from the father's asthma, eczema, urticaria, or allergic rhinitis. It is also notable that sex did not influence baseline lung function or bronchial responsiveness, contrasting with previous study reports of reduced lung function in males as a cause for wheezing disorder being more common in boys.<sup>42</sup> This should encourage further research on the sex differences in wheezing disorders.

We saw no influence on baseline lung function and bronchial responsiveness in the newborns from parents' age; mother's previous deliveries; social status; mother's use of alcohol, antibiotics, steroids, or acetylsalicylic acid; pregnancy complications; mother's asthma status in the third trimester; mode of delivery; Apgar score; or bacterial colonization the airways of the newborn.

The parabola development of bronchial responsiveness peaking at 3 months of age is a novel finding. The median age for hospitalization for acute viral bronchiolitis coincides with this peak in bronchial hyperresponsiveness, suggesting the physiologic background for the exaggerated bronchial obstruction in response to viral infection. However, this observation needs confirmation from repeated measurements within the same infants.

## Future research

The association between BMI and lower baseline lung function and the association between paracetamol and bronchial hyperresponsiveness in neonates need confirmation in future studies, and the mechanisms should be explored.

Despite this comprehensive search for prenatal factors influencing the innate lung function, the prediction model identified describes only 34% of the variation in baseline FEV<sub>0.5</sub> and 5% of the variation in bronchial responsiveness, suggesting that besides genetics, other as yet undisclosed exposures have a strong influence, particularly on bronchial responsiveness, and the main factors determining neonatal lung function remains elusive.

## Conclusion

The mother's smoking was associated with adverse effects on lung function even after extensive confounder adjustment. Importantly, baseline lung function was negatively associated with BMI, suggesting that the accumulating evidence associating body proportion and wheezing disorders may be a result of shared genes or prenatal nutrition.

We thank the children and parents participating in the COPSAC cohort as well as the COPSAC study team.

## Key messages

- Neonatal lung function is reduced from high BMI in the newborn and the mother's smoking in pregnancy.
- Notably the neonatal lung function was not affected by sex, parents' atopy, or bacterial colonization of the airways.

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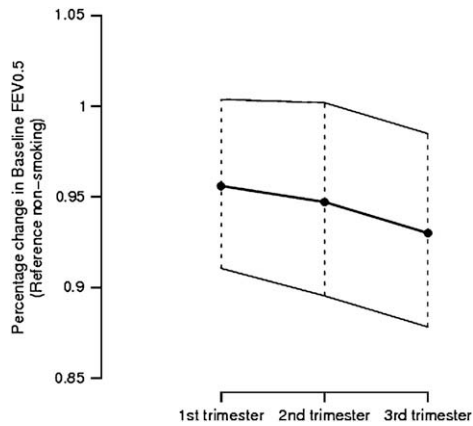


**FIG E1.** Percentage change in baseline FEV<sub>0.5</sub> in mothers smoking in first, second, and third trimester. Adjusted for age at test and BMI and weight at birth.

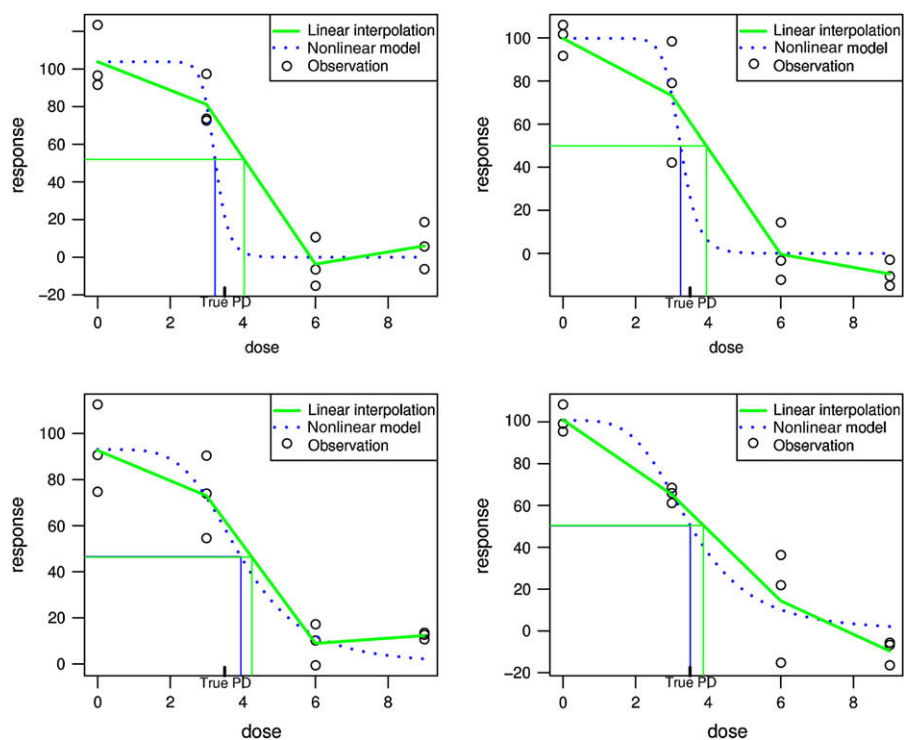


**FIG E2.** Four examples comparing provocative dose (PD) estimates by using the traditional linear interpolation or the logistic model.

TABLE E1. Univariate risk factor analyses adjusted for lifespan at examination date and length at birth

|                                                               | Total | Yes | Baseline FEV <sub>0.5</sub> |              |              |            | PD <sub>15</sub> (PTcO <sub>2</sub> ) |              |              |            |
|---------------------------------------------------------------|-------|-----|-----------------------------|--------------|--------------|------------|---------------------------------------|--------------|--------------|------------|
|                                                               |       |     | Estimate                    | CI 2.5%      | CI 97.5%     | P value    | Estimate                              | CI 2.5%      | CI 97.5%     | P value    |
| Demographics                                                  |       |     |                             |              |              |            |                                       |              |              |            |
| Male                                                          | 411   | 203 | 0.983                       | 0.943        | 1.024        | .42        | 0.824                                 | 0.576        | 1.179        | .29        |
| Nonwhite                                                      | 411   | 14  | 1.044                       | 0.926        | 1.177        | .48        | 0.808                                 | 0.289        | 2.257        | .81        |
| Father's age at birth of proband                              | 401   |     |                             |              |              |            |                                       |              |              |            |
| <25 y                                                         | 29    |     | Reference                   |              |              |            |                                       |              |              |            |
| 25-35 y                                                       | 259   |     | 0.993                       | 0.916        | 1.076        | .85        | 1.419                                 | 0.689        | 2.923        | .34        |
| ≥35 y                                                         | 113   |     | 0.979                       | 0.899        | 1.067        | .63        | 1.102                                 | 0.512        | 2.370        | .80        |
| Mother's age at birth of proband                              | 411   |     |                             |              |              |            |                                       |              |              |            |
| <25 y                                                         | 52    |     | Reference                   |              |              |            |                                       |              |              |            |
| 25-35 y                                                       | 290   |     | 0.971                       | 0.912        | 1.034        | .36        | 1.283                                 | 0.737        | 2.234        | .38        |
| ≥35 y                                                         | 69    |     | 0.959                       | 0.888        | 1.035        | .28        | 1.530                                 | 0.781        | 2.997        | .21        |
| Previous deliveries                                           | 368   |     |                             |              |              |            |                                       |              |              |            |
| 0                                                             | 217   |     | Reference                   |              |              |            |                                       |              |              |            |
| 1                                                             | 114   |     | 0.956                       | 0.912        | 1.003        | .07        | 1.492                                 | 0.977        | 2.280        | .06        |
| 2+                                                            | 37    |     | 1.046                       | 0.971        | 1.128        | .24        | 0.909                                 | 0.466        | 1.770        | .78        |
| Occupation, mother                                            | 384   |     |                             |              |              |            |                                       |              |              |            |
| Nonprofessional                                               | 128   |     | Reference                   |              |              |            |                                       |              |              |            |
| Professional                                                  | 175   |     | 1.002                       | 0.956        | 1.051        | .93        | 0.890                                 | 0.586        | 1.350        | .58        |
| Student                                                       | 42    |     | <b>1.091</b>                | <b>1.015</b> | <b>1.172</b> | <b>.02</b> | 0.828                                 | 0.427        | 1.606        | .58        |
| Unemployed                                                    | 39    |     | 1.031                       | 0.957        | 1.110        | .43        | 0.544                                 | 0.283        | 1.044        | .07        |
| Highest education, mother                                     | 384   |     |                             |              |              |            |                                       |              |              |            |
| At most college                                               | 234   |     | Reference                   |              |              |            |                                       |              |              |            |
| Medium                                                        | 99    |     | 0.990                       | 0.942        | 1.040        | .67        | 1.069                                 | 0.698        | 1.640        | .76        |
| University                                                    | 51    |     | 0.998                       | 0.937        | 1.064        | .96        | 0.902                                 | 0.522        | 1.559        | .71        |
| Income (Danish Kroner)                                        | 394   |     |                             |              |              |            |                                       |              |              |            |
| <400,000                                                      | 112   |     | Reference                   |              |              |            |                                       |              |              |            |
| 400,000-600,000                                               | 182   |     | 0.980                       | 0.933        | 1.029        | .41        | 1.170                                 | 0.756        | 1.810        | .48        |
| >600,000                                                      | 90    |     | 0.970                       | 0.915        | 1.027        | .29        | 0.912                                 | 0.552        | 1.507        | .72        |
| Atopic predisposition                                         |       |     |                             |              |              |            |                                       |              |              |            |
| Mother                                                        |       |     |                             |              |              |            |                                       |              |              |            |
| Allergic rhinitis                                             | 410   | 308 | 0.992                       | 0.947        | 1.040        | .74        | 0.895                                 | 0.595        | 1.345        | .59        |
| Atopic dermatitis                                             | 411   | 196 | 0.994                       | 0.954        | 1.036        | .78        | 0.795                                 | 0.559        | 1.131        | .20        |
| Urticaria                                                     | 409   | 169 | 1.031                       | 0.989        | 1.075        | .15        | 0.719                                 | 0.505        | 1.025        | .07        |
| Father                                                        |       |     |                             |              |              |            |                                       |              |              |            |
| Asthma                                                        | 398   | 65  | 1.012                       | 0.957        | 1.070        | .67        | 1.094                                 | 0.673        | 1.777        | .72        |
| Allergic rhinitis                                             | 396   | 124 | 1.000                       | 0.956        | 1.045        | .98        | 1.073                                 | 0.725        | 1.587        | .72        |
| Atopic dermatitis                                             | 396   | 52  | 0.976                       | 0.919        | 1.037        | .44        | 1.251                                 | 0.735        | 2.127        | .41        |
| Urticaria                                                     | 390   | 56  | 0.979                       | 0.923        | 1.038        | .48        | 1.081                                 | 0.635        | 1.839        | .77        |
| Third trimester exposures                                     |       |     |                             |              |              |            |                                       |              |              |            |
| Smoking                                                       | 411   | 63  | <b>0.935</b>                | <b>0.883</b> | <b>0.990</b> | <b>.02</b> | 0.710                                 | 0.431        | 1.171        | .18        |
| Alcohol                                                       | 411   | 73  | 0.953                       | 0.904        | 1.005        | .08        | 1.470                                 | 0.913        | 2.367        | .11        |
| Acetylsalicylic acid and nonsteroidal anti-inflammatory drugs | 411   | 6   | 1.037                       | 0.875        | 1.229        | .68        | 0.767                                 | 0.164        | 3.584        | .74        |
| Paracetamol                                                   | 411   | 59  | 0.981                       | 0.926        | 1.040        | .52        | <b>0.562</b>                          | <b>0.344</b> | <b>0.919</b> | <b>.02</b> |
| Systemic steroid                                              | 411   | 5   | 1.046                       | 0.870        | 1.259        | .63        | 0.613                                 | 0.113        | 3.344        | .57        |
| Antibiotics                                                   | 411   | 58  | 1.011                       | 0.954        | 1.03         | .69        | 1.239                                 | 0.727        | 2.110        | .43        |
| Third trimester complications                                 |       |     |                             |              |              |            |                                       |              |              |            |
| Hemorrhage                                                    | 375   | 4   | 1.049                       | 0.856        | 1.284        | .65        | 0.311                                 | 0.057        | 1.697        | .18        |
| Preterm contractions                                          | 371   | 246 | 0.972                       | 0.929        | 1.017        | .22        | 1.396                                 | 0.933        | 2.086        | .10        |
| Pre-eclampsia                                                 | 375   | 20  | 0.959                       | 0.874        | 1.053        | .38        | 1.307                                 | 0.533        | 3.204        | .56        |
| Pneumonia                                                     | 411   | 31  | 1.059                       | 0.979        | 1.147        | .15        | 1.192                                 | 0.599        | 2.369        | .62        |
| Influenza                                                     | 411   | 49  | 1.006                       | 0.944        | 1.072        | .85        | 1.579                                 | 0.913        | 2.731        | .10        |
| Mother's asthma                                               | 410   |     |                             |              |              |            |                                       |              |              |            |
| Better                                                        | 113   |     | Reference                   |              |              |            |                                       |              |              |            |
| Unchanged                                                     | 213   |     | 0.996                       | 0.949        | 1.045        | .87        | 1.074                                 | 0.714        | 1.617        | .73        |
| Worse                                                         | 84    |     | 1.042                       | 0.982        | 1.106        | .18        | 1.286                                 | 0.769        | 2.151        | .34        |
| Newborn                                                       |       |     |                             |              |              |            |                                       |              |              |            |
| Delivery                                                      | 411   |     |                             |              |              |            |                                       |              |              |            |
| Natural                                                       | 273   |     | Reference                   |              |              |            |                                       |              |              |            |
| Vacuum                                                        | 53    |     | 1.003                       | 0.943        | 1.066        | .93        | 0.958                                 | 0.566        | 1.623        | .87        |
| Cesarean                                                      | 85    |     | 1.019                       | 0.968        | 1.073        | .96        | 1.250                                 | 0.802        | 1.947        | .32        |
| Apgar score ≥9 at 5 minutes                                   | 405   | 363 | 0.978                       | 0.914        | 1.046        | .52        | 1.478                                 | 0.845        | 2.584        | .17        |
| BMI at birth (kg/m <sup>2</sup> )                             | 411   |     |                             |              |              |            |                                       |              |              |            |
| 7-12                                                          | 110   |     | Reference                   |              |              |            |                                       |              |              |            |
| 12-13                                                         | 132   |     | 1.045                       | 0.990        | 1.102        | .11        | 0.987                                 | 0.618        | 1.576        | .96        |
| 13-14                                                         | 96    |     | <b>1.073</b>                | <b>1.012</b> | <b>1.138</b> | <b>.02</b> | 0.951                                 | 0.571        | 1.585        | .85        |
| 14-17                                                         | 73    |     | 1.016                       | 0.954        | 1.082        | .11        | 1.373                                 | 0.798        | 2.362        | .25        |

Boldface and italics indicate the significant variables.

**TABLE E2.** Comparison of PD<sub>15</sub>(PTcO<sub>2</sub>) obtained by interpolation and by a nonlinear logistic model

| Dose-step | N   | Geometric mean PD <sub>15</sub> (PTcO <sub>2</sub> ) (N) by logistic model | Geometric mean PD <sub>15</sub> (PTcO <sub>2</sub> ) (N) by interpolation | Geometric mean ratio new/old (95% CI) |
|-----------|-----|----------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------|
| 1         | 1   | —                                                                          | —                                                                         | —                                     |
| 3         | 4   | —                                                                          | 0.074 (4)                                                                 | —                                     |
| 4         | 62  | 0.055 (56)                                                                 | 0.062 (60)                                                                | 0.979 (0.680-1.409)                   |
| 5         | 133 | 0.182 (130)                                                                | 0.192 (133)                                                               | 1.005 (0.908-1.111)                   |
| 6         | 118 | 0.617 (118)                                                                | 0.602 (118)                                                               | 1.025 (0.880-1.194)                   |
| 7         | 55  | 1.281 (53)                                                                 | 0.957 (55)                                                                | 1.486 (0.983-2.247)                   |
| 8         | 6   | 7.871 (6)                                                                  | 5.177 (6)                                                                 | 1.520 (0.972-2.377)                   |
| Total     | 379 | 0.319 (363)                                                                | 0.302 (376)                                                               | 1.074 (0.968-1.191)                   |

The PD<sub>15</sub>(PTcO<sub>2</sub>) estimated by interpolation is systematically lower than PD<sub>15</sub>(PTcO<sub>2</sub>) estimated by logistic modeling in children tolerating repeated dose-steps.

