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Interaction between Asthma and Lung Function Growth in Early Life

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At a glance commentary: The lung function deficit associated with asthma may precede or develop secondary to disease. The causal direction behind this association is important for the focus of preventive measures and research into the origins of asthma. This study suggests that children developing asthma by age 7 have lung function deficit and increased bronchial responsiveness as neonates. This lung function deficit seems to progress to age 7. Therefore research into the origins and prevention of asthma should consider early life both before and after birth.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

Data from this manuscript has not been presented before in abstract or any other form.

ABBREVIATIONS

Neonatal-$FEV_{0.5}$	Forced expiratory volume after 0·5 second in neonates
Neonatal-FEF_{50}	Forced expiratory flow at 50% of vital capacity second in neonates
Neonatal-$PD_{15}(TcO_2)$	Bronchial responsiveness to methacholine assessed in neonates by TcO_2
Child-FEV_1	Forced expiratory volume after 1 second at age 7
Child- FEF_{50}	Forced expiratory flow at 0·5 second in neonates at age 7

ABSTRACT

Rationale

The causal direction between asthma and lung function deficit is unknown, but important for the focus of preventive measures and research into the origins of asthma.

Objectives

To analyze interaction between lung function development and asthma from birth to 7 years of age.

Methods

The Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) is a prospective clinical study of a birth-cohort of 411 at-risk children. Spirometry was completed in 403 (98%) neonates and again by age 7 in 317 children (77%).

Measurements

Neonatal spirometry and bronchial responsiveness to methacholine was measured during sedation by forced flow-volume measurements. Asthma was diagnosed prospectively from daily diary cards and 6-monthly clinic visits.

Main Results

Children with asthma by age 7 (14%) already had a significant airflow deficit as neonates (FEF_{50} reduced by 0·34 Z-score by 1 month, p=0·03). This deficit progressed significantly during early childhood (FEF_{50} reduced by 0·82 Z-scores by age 7, p<0·0001), suggesting that approximately 40% of the airflow deficit associated with asthma is present at birth while 60% develops with clinical disease. Environmental tobacco exposure, but not allergic sensitization, also hampered airflow growth.

Bronchial responsiveness to methacholine in the neonates was associated with the development of asthma, p=0.01.

Conclusions

Children developing asthma by age 7 had a lung function deficit and increased bronchial responsiveness as neonates. This lung function deficit progressed to age 7. Therefore research into the origins and prevention of asthma should consider early life both before and after birth.

Abstract word count: 243

Key-words: Asthma, lung function, young child, infant, neonate

INTRODUCTION

Children with asthma have reduced lung function by early school age. Thereafter the lung function seems to track at a fixed percentile.[1-7] It is the important research question whether the loss of lung function associated with asthma is mainly a cause or a consequence of the disease. Are children born with abnormal lung function and thereby programmed for development of asthma or is the loss of lung function from asthma a consequence of the disease process during the symptomatic years? These questions are important for the direction of research into the origins and prevention of asthma; i.e. should we expect genetic or prenatal programming of the disease; or is there an early window of opportunity to prevent airway remodelling during early symptoms?

The available evidence is limited and contradictory on the relation between neonatal lung function and development of asthma and other wheezy disorders. Some birth cohort studies suggested that lung function deficit in neonates preceded childhood asthma.[8;9] Others found that infants who developed persistent wheeze had normal infant lung function but significantly reduced spirometry by age 6.[10] Likewise the evidence is ambiguous on the association between infant bronchial responsiveness and development of asthma.[8;11-13]

We analysed the interaction between asthma and lung function growth from neonatal age to age 7 in the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) birth cohort.

Data from this manuscript has not been presented before in abstract or any other form.

METHODS

See Appendix for further details

COPSAC Cohort

The Copenhagen Prospective Study on Asthma in Childhood (COPSAC) is a prospective clinical study of a birth-cohort of 411 children. All mothers had a history of doctor's diagnosis of asthma after age 7. The newborns were enrolled in the first month of life; key exclusion criteria were severe congenital abnormality, gestational age <36 weeks and lung symptoms prior to enrolment as previously described in detail.[14-16]

The study was approved by the Ethics Committee for Copenhagen (KF 01-289/96) and the Danish Data Protection Agency (2008-41-1754). Written informed consent was obtained from both parents.

Spirometry and bronchial responsiveness to metacholine

Neonatal spirometry and bronchial responsiveness to methacholine was obtained by forced flow-volume measurements applying the raised volume rapid thoracic compression technique as previously described in a sequence of studies.[17-19] Lung function was measured by spirometry in the child's 7th year of life using a pneumotachograph Masterscope Pneumoscreen, system 754916 spirometer (Erich Jäeger, Würtzburg, Germany).

FeNO Measurements

Baseline FeNO was measured at 7 years of age by an online technique[20] using NIOX® FLEX (Aerocrine, Solna, Sweden) in accordance with international recognized guidelines[21]

Clinical follow-up

Participants were assessed at six–monthly intervals at the COPSAC clinical research unit and additional visits were arranged immediately upon the onset of respiratory symptoms. At every visit, the child was given a full physical examination. The doctors employed at the clinical research unit were acting primary physician for the children of the cohort for diagnosis and treatment of any respiratory symptoms. Asthmatic symptoms were recorded in daily diaries.[22]

Asthma

Asthma was diagnosed based on the following 4 mandatory criteria (1) recurrent episodes of troublesome lung symptoms recorded in the daily dairy cards as five episodes within 6 months, each episode lasting at least three consecutive days; (2) symptoms typical of asthma (recently termed "multi-trigger wheeze"[23]) based on doctors interviews of the parents at the clinical research unit. Such symptoms included exercise induced symptoms; prolonged nocturnal cough; recurrent cough outside common cold; symptoms causing wakening at night; (3) need for intermittent rescue use of inhaled β_2-agonist; and (4) response to a 3-month course of inhaled corticosteroids and relapse when stopping treatment.

Treatment Algorithm

Medical treatment for asthma followed strict predefined algorithms as detailed in the online appendix.

Temporal categories of asthmatic symptoms

Temporal categories were based on the diary-registered asthmatic symptoms in the first 6 years of life. The individual average asthma symptom load index was calculated as the sum of days with symptoms in different age intervals. Categorization into 3 year intervals and further

into the 3 categories of transient early, late onset and persistent symptoms seemed most appropriate as described in details previously[22] and in the online appendix.

Atopic traits

Allergic sensitization against common inhalant allergens (cat, dog, horse, birch, timothy grass, mugwort, *D. pteronyssinus* , *D. farina*, and moulds) was determined at age 6 months, 18 months, 4 years and 6 years by skin prick test (SPT) (allergen extracts from ALK-ABELLÓ, Copenhagen, Denmark) and measurement of serum specific IgE (ImmunoCAP assay, Pharmacia Diagnostics AB, Uppsala, Sweden).[24] Sensitization was defined as a positive SPT and/or specific IgE test for any of the tested allergens and was analyzed as a dichotomized measurement. Cut off levels used specific-IgE ≥ 0.35 IU/mL and SPT mean weal diameter >= 2mm larger than the negative control at age 6 and 18 months and specific-IgE ≥ 3.5 IU/mL and SPT>= 3mm at 4 years and 6 years. Sensitization was defined as a positive SPT and/or specific IgE test for any of the tested allergens and was analyzed as a dichotomized measurement.

Total IgE level was measured by the ImmunoCAP assay, detection limit was 0.1 IU/mL.

Blood eosinophil count (10^9 cells per litre) was assessed at age 6 years.

Atopic dermatitis was diagnosed by using the Hanifin-Rajka criteria as detailed in previous reports. [25-28] Eczema was categorized as ever having the diagnosis before 7 years of age.

Allergic rhinitis was defined by symptoms and relevant allergic sensitization as previously described[29]

Statistical Analyses

Neonatal-$FEV_{0.5}$ and neonatal-FEF_{50} were calibrated by BMI, weight and lifespan at test and the neonatal bronchial responsiveness to methacholine were calibrated by weight, lifespan and lifespan^2 at test in accordance with our previous analysis of determinants for the neonatal lung function.[19] Spirometry by age 7 was calibrated by height and gender.

Analyses of association between neonatal lung function and asthma were adjusted by gender and smoking during third trimester of pregnancy.

The provocative dose for a 15% decrease in PtcO2 (neonatal-$PD_{15}(TcO_2)$) was estimated by fitting each infant's dose response curve with a non-linear logistic function as previously detailed.[19] Log-transformation of the neonatal-$PD_{15}(TcO_2)$ estimates assured normal distribution.

The association between neonatal lung function and the cross-sectional diagnosis of current asthma at 7 years of age was investigated by logistic regression. Wald 95% confidence intervals of odds ratios were calculated on a log-scale and back-transformed. *P*-values correspond to Wald tests.

Lung function growth was investigated in a generalized linear regression model analysing lung function at 7 years adjusted for neonatal lung function. The effect of asthma, temporal categories of asthmatic symptoms, allergic sensitization, blood eosinophil levels and atopic dermatitis on lung function growth was investigated in this model adjusting for environmental tobacco exposure (ETS) assessed by nicotine in the hair of the child at 3 years of age[30].

Lung function measures were illustrated by their internal z-score values.

All continuous covariates were standardized by the inter quartile range (IQR).

All analyses were done using SAS version 9·2(SAS Institute Inc, Cary, NC) and the open source statistical programming environment R version 2.11.0(www.r-project.org).

RESULTS

Lung Function Completion

Neonatal spirometry was measured at 1 month in 403 neonates (98%) and neonatal-PD15(TcO2) in 362 neonates (88%) from the birth cohort of 411.[17-19] Spirometry by age 7 was measured in 317 children (77%).

Asthma by age 7

Forty-seven of 336 children (14%) had asthma by age 7. 67% of the children with asthma were on current treatment with inhaled corticosteroid at the time of lung function measurement. Children with asthma had higher prevalence of allergic rhinitis, and blood eosinophil levels, and atopic dermatitis, while FeNO was not significantly increased. Sensitization to inhaled allergens was approximately twice as common at all ages in children with asthma. (Table 1) There was no difference with respect to total IgE levels, gender, race, ETS exposure or household income between the two groups.

Association between Neonatal Lung Function and Asthma by age 7

Neonatal Airflow

A lower level of neonatal-FEF_{50} was significantly associated with asthma by age 7; OR 1.57 [CI 1·04;2·37], p=0·03; i.e. an inter-quartile reduction in neonatal-FEF_{50} lead to 57% increased risk of asthma by age 7 (Table 2). Correspondingly, neonatal-FEF_{50} was significantly reduced in children who later developed asthma compared to children who did not develop asthma (z-score difference -0·34, p=0·03). Similar trends were seen for neonatal-$FEV_{0.5}$.

Neonatal bronchial responsiveness

Neonatal bronchial responsiveness (low Neonatal-$PD_{15}(TcO_2)$) was significantly associated with asthma by age 7 (OR 1·59 [1·11;2·28], p=0·01) Likewise, Neonatal-$PD_{15}(TcO_2)$ was significantly reduced in children who had developed asthma by age 7 years compared to children who did not develop asthma (z-score difference -0·48, p=0·005) (Table 2).

Asthma and Lung Function Growth

Child-FEF_{50}, Child-FEV_1, Child-FVC and Child FEV_1/FVC were all significantly reduced in children with current asthma compared to those without (Table 3).

The neonatal airflow deficit associated with asthma progressed from a neonatal-FEF_{50} Z-score deficit of 0·34 (p=0·03) (Table 2) to a child-FEF_{50} Z-score deficit of 0·82 (p<0·0001) at age 7 years (Table 3) (Fig 1A); i.e. the airflow deficit in neonatal life constituted 41% of the deficit at 7 years of age.

This progressing airflow deficit was analysed in a multivariable model of child-FEF_{50}, with neonatal-FEF_{50} included as covariate (Table 4). Neonatal- FEF_{50} and child-FEF_{50} were significantly associated. Asthma by age 7 had an independent negative effect on airflow growth (-0·69 z-score [-1·01;-0·37] p<0·0001), meaning that for children with similar infant airflow, development of asthma was associated with airflow reduction of 0.69 z-score by age 7. ETS assessed at 3 years also had an independent negative effect on airflow growth.

A similar trend of an effect from asthma was seen on neonatal-$FEV_{0.5}$ /. child-FEV_1 (Figure 1B) with an increasing lung function deficit from Z-score -0·29 in neonatal life to -0·73 at age 7 years (neonatal deficit constituting 42% of the deficit at 7 years) although the association in neonatal life failed to reach statistical significance. There was a significant independent effect

of asthma on airflow growth, measured as effect of asthma on child-FEV1 adjusted for neonatal-FEV0.5 (Table 4).

Atopy and Lung Function growth

Child-FEF_{50}, Child-FEV_1, Child-FVC and Child FEV_1/FVC were reduced similarly in children with asthma with or without allergic sensitization at 6 years of age (Table 3).

In multivariable models of lung function growth, neither allergic sensitization, blood eosinophil level nor atopic dermatitis affected lung function growth independently of asthma (table 1-online). Also, none of these atopic traits modified the effect of asthma on lung function growth (p-values for interaction > 0·18, data not shown).

Temporal categories of asthmatic symptoms and Lung Function Growth

Figure 1-online suggests a lung function deficit at birth progressing to age 7 years for children with persistent symptoms but not transient early or late onset symptoms, which was confirmed in the multivariable analyses of lung function growth (Table 2-online).

DISCUSSION

Principal findings of the study

Children who developed asthma by age 7 had reduced airflow and increased bronchial responsiveness as neonates. This airflow deficit progressed in the first 7 years of life suggesting that disease mechanisms are operating both before and after birth, and that research into the origins and prevention of asthma should include pre- and perinatal life, but also that a possible window of opportunity for preventing further loss of lung function may exist in the first years of life.

Strength and weaknesses of the study

This is the most comprehensive prospective study of the association between early childhood asthma and lung function changes from birth to school age. Lung function measurements were completed during sedation in 404 of 411 asymptomatic neonates under standardized conditions in this single-centre study.[17-19]

The cohort was studied within a narrow age-range 1 month after birth and were not included if they had any lung symptoms prior to the test. Therefore it seems unlikely that loss of lung function could have happened during the first weeks of life but is more likely a reflection of prenatal differences.

The lung function measurements were based on comprehensive analyses in preparation for this study.[17-19] Neonatal lung function indices were chosen based on sensitivity in response to metacholine challenge[17;18]. The 3 most sensitive measures were PTcO2, FEV0.5 and FEF50, and these were therefore chosen for the present study. The specific focus of this study was to investigate if the lung function deficit associated with early school age asthma takes place pre-

or postnatally. This can only be studied with a measure of lung function in neonates that reflects the same physiological changes as school age lung function. Significant correlation with school age lung function is therefore an indicator of the relevance of an infant lung function measure for this purpose. As seen in the multi-variable models of school-age lung function (Table 4), neonatal-FEF50 was strongly associated with school-age FEF50 while only a borderline significant effect was seen for FEV0.5/FEV1, suggesting that FEF50 is the most relevant measure with the aim of the current study. This is actually to be expected since the initial selection[17] was based on comparison of sensitivity to methacoline challenges (mainly central obstruction), while early asthma pathology is suspected to predominant in the peripheral airways. Likewise FEV0.5 is probably more influenced from central airways resistance while and FEF50 is suspected to be a better reflection of peripheral resistance. In line with this, all associations in the study were strongest for this measure.

We used infant spirometry adapted as the state-of-the-art raised volume rapid thoraco-abdominal compression technique, which provides volume-anchored flow measures and adjusted for internally identified determinants of neonatal lung function.[19]

The diagnosis of asthma was based on close monitoring of all study participants by daily diary cards and visits to the research clinic every 6 months and at every acute symptom during the first 7 years of life. At these clinic visits the symptom diary and the clinical history was evaluated by doctors with paediatric training all working from the same standard operating procedures. Diagnosis was based on algorithms that were defined prior to this study.[16;22] The diagnostic algorithm standardizes a minimum severity of symptoms assessed by symptom frequency and duration; it assured consideration of differential diagnoses, and emphasized a diagnosis of asthma disregarding sensitization or other atopic stigmata. By this definition we

prioritize diagnostic specificity rather than sensitivity, i.e, a homogenous population of childhood asthma rather than the common heterogenous population of community diagnosed cases. Since the spectrum of asthma in children represent a heterogeneous group of diseases, our results may not be extrapolated to children with milder asthmatic symptoms or diagnosed using a more heterogeneous asthma definition.

The external validity of the conclusions of this study is limited by the cohort selection of pregnant mothers with a history of asthma, mainly white subjects, excluding preterm newborns.

Meaning of the study

By age 7 children with asthma had a significant lung function deficit. This is in agreement with longitudinal studies of spirometry in asthma showing lung function deficit at school age with no further progression in adolescence and adulthood.[1-7]

Previous evidence on the association between infant spirometry and this loss of lung function in asthmatic children is ambiguous due to few studies of limited size and with methods of little standardization, as recently reviewed.[31] The Perth birth cohort study of 157 children suggested that persistent wheeze at both 4-6 years and 11 years of age was associated with lung function deficit in neonates, but did not progress with time, suggesting that impaired infant lung function may have predetermined the development of persistent wheezy disorders with no progression in preschool years.[8] In contrast the Tucson Children's Respiratory Study reported that infants who developed persistent wheeze had normal infant lung function but significantly reduced spirometry by age 6,[10] suggesting that the lung function deficit was caused by the persistent wheezy disorder in preschool years. In their study children with persistent wheeze had a non-significant reduction in infant lung function

of approximately 0·3 z-score compared to children who never wheezed. We found a similar effect size albeit significant for both school-age asthma and the category of persistent symptoms, suggesting that the conclusion from the Tucson cohort was due to limited statistical power (125 children with infant lung function measurement). Noteworthy, the Tucson cohort used infant spirometry without volume anchoring (i.e. before the "pump-up method") as an important methodological difference from our method which is anchored by a predefined airway pressure before the compression.

Another study reported association between neonatal tidal breathing patterns, neonatal respiratory-system compliance and asthma at 10 years of age.[9] None of these tidal breathing patterns were associated with lung function at school age suggesting that they represent some other pathology than abnormal lung function and therefore do not provide evidence on the temporal relationship between asthma symptoms and lung function deficit.

It is interesting that neonatal airway reactivity was a stronger predictor of asthma than neonatal lung function. This may suggest an ongoing disease process that has not yet caused lung function changes. The association between infant bronchial responsiveness and development of asthma has been studies in 2 other cohorts. In the Perth cohort there was an association with asthma at age 6 years (follow-up in 95 of 253 children),[11] but not by age 11 years (follow-up in 185 of 253 children[8]. A British cohort of 73 children found association with wheeze in infancy but not at 10 years of age) – ref clarke og Wilson [8] , [12;13]. The discrepancy between the current results and some previous studies may be related to limited statistical power in relatively small studies or the marked differences in both exposure and outcome assessments between studies. Bronchial responsiveness may also be stronger associated with asthma earlier in life than later.

Neither allergic sensitization, atopic dermatitis nor blood eosinophil level affected lung function development independently of asthma symptoms. This contrasts one previous study suggesting that only children with early allergic sensitization, and particularly those with high allergen exposure, have reduced lung function at school age.[7] However, we assessed the independent effect of sensitization for infant with similar lung function at birth, which was not adjusted for in the previous study. Furthermore, we used a very specific algorithm based diagnosis of asthma assuring similar severity of asthma in all asthma-diagnosed children. It is possible that some of the non-sensitized children in the previous cohort had less severe asthma and therefore normal lung function. Finally, only 7% of our children were sensitized to inhalant allergens at 18 months, and interaction with high allergen exposure would only affect a small minority of children, while other mechanism are driving the loss of lung function in the majority of children. Therefore, research into lung function loss should not be restricted to atopy-related mechanisms.

Approximately 40% of the lung function deficit associated with asthma by age 7 (and "persistent wheeze") was already present at birth. This is in keeping with the hypothesis of early programming of asthma suggesting that prenatal mechanisms should be targeted in order to prevent lung function loss.

The progressive loss of lung function after birth associated with asthma suggests a potential window of opportunity after birth, and modifying the airway remodeling during early childhood has been a major focus of asthma research. The available randomized controlled trials show that inhaled corticosteroids cannot modify the natural course of lung function impairment associated with childhood asthma, whether given to more severe asthma,[32] mild asthma[33] or intermittent wheeze.[15] The present study supports this by demonstrating a

progressive loss of lung function from birth to 7 years of age despite a very careful monitoring of the cohort with rigid algorithms for very early treatment with inhaled corticosteroid. The present study may also *explain* the lack of effect from early intervention with inhaled corticosteroids. First, asthma treatment is initiated after onset of symptoms while our study suggests that a significant loss of lung function already took place before onset of symptoms, indicating irreversible changes and/or a process that cannot be modified at the symptomatic stage. Second, steroids are most effective against eosinophillic airway inflammation, while our findings suggests that lung function loss is independent of sensitization or increased eosinophil levels. Drugs targeting other pathways may therefore be needed.

ETS had an independent negative influence on airflow growth from neonates to age 7. We previously demonstrated in the same birth cohort a neonatal lung function deficit if mothers smoked during pregnancy. This shows a detrimental environmental factor operating both before and after birth. Interestingly, this independent association suggests that ETS can also cause damage to the airways in the absence of symptomatic airway inflammation.

Conclusion

Children developing asthma by age 7 had lung function deficit and increased bronchial responsiveness as neonates suggesting that research should consider prenatal programming in search of the origins and prevention of asthma. The lung function deficit progressed to age 7 indicating a potential window of opportunity for early clinical intervention.

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Figure legends

Figure 1: Forced expiratory flow rate at 1 month and 7 years of age in children with (dashed line) and without (solid line) current asthma by age 7 years. Mean Z-score and 95% CI.

Table 1: Cohort description according to asthma status by age 7 years.

	No Asthma	Asthma	p-value
N	289	47	
Girls	50% (144/289)	49% (23/47)	0.91*
Caucasians	97% (280/289)	98% (46/47)	0.71**
Nicotine in hair at 3 years ng/mg (median (IQR)) (N)	0.240 (0.105-1.038) (N=252)	0.228 (0.110-0.822) (N=42)	0.98***
Household income (Low/Average/High)	26% (73/281) 48% (136/281) 26% (72/281)	26% (12/46) 50% (23/46) 24% (11/46)	0.97*
Atopic traits			
Sensitized to inhalant allergens 6 and/or 18 months	7% (20/286)	13% (6/47)	0.17*
Sensitized to inhalant allergens 4 yrs	11% (29/263)	24% (11/45)	0.01*
Sensitized to inhalant allergens 6 yrs	17% (45/258)	30% (14/47)	<0.05*
Total IgE 6 yrs (Median (IQR)) (N)	46.4 (18.7 - 107.7) (249)	33.9 (15.6 - 178.3) (43)	0.95[ff]
B-eosinophils 6 yrs (Median (IQR)) (N)	0.320 (0.170 - 0.520) (235)	0.455 (0.335 - 0.615) (40)	0.02[ff]
Atopic dermatitis (ever)	48% (128/267)	63% (29/46)	0.06*
Allergic rhinitis	11% (28/245)	23% (10/43)	0.03*
FeNO N (Median (IQR))	8.9 (7.1-12.25) (256)	10.7 (7.2-17.0) (47)	0.17***

*Chisq, ** Fisher's exact test, ***Kruskal-Wallis, [f]T-test, [ff]Log transformed before test. Continuous variables are presented as Median (IQR). Categorical variables are presented as % (n/total).

Table 2: Association between neonatal lung function measures and asthma at age 7 years.

Neonatal lung function	***Risk of asthma**** *OR per IQR*	***Neonatal lung function z-score**** *Difference between children with or without asthma by age 7.*
Neonatal-FEF_{50}	1.57 [1.04;2.37]; p=0.03	-0.34; p=0.03
Neonatal-$FEV_{0.5}$	1.41 [0.94;2.10]; p=0.09	-0.29; p=0.07
Neonatal-$PD_{15}(TcO_2)$	1.59 [1.11;2.28]; p=0.01	-0.48; p=0.005

* Analyses are adjusted by gender and smoking during third trimester of pregnancy

Table 3: Spirometry by age 7 according to asthma status.

	No Asthma	Asthma	Asthma + sensitization	Asthma + no sensitization
Child-FEF_{50} (L/S)	2.05 ± 0.48	1.64 ± 0.41 (p<0.0001)	1.70 ± 0.36 (p=0.008)	1.62 ± 0.44 (p<0.0001)
Z-score (Mean ± std)	0.12 ± 0.98	-0.70 ± 0.83	-0.59 ± 0.72	-0.75 ± 0.88
N	(264)	(44)	(14)	(30)
Child-FEV_1 (L)	1.49 ± 0.18	1.36 ± 0.21 (p<0.0001)	1.36 ± 0.13 (p=0.01)	1.36 ± 0.24 (p<0.0001)
Z-score (Mean ± std)	0.12 ± 0.94	-0.61 ± 1.11	-0.575 ± 0.704	-0.62 ± 1.25
N	(267)	(46)	(14)	(32)
Child-FVC (L)	1.61 ± 0.19	1.52 ± 0.22 (p=0.008)	1.53 ± 0.18 (p=0.15)	1.52 ± 0.23 (p=0.02)
Z-score (Mean ± std)	0.06 ± 0.96	-0.35 ± 1.07	-0.33 ± 0.90	-0.36 ± 1.14
N	(276)	(46)	(14)	(32)
Child FEV1/FVC	0.95 (0.91-0.99)	0.90 (0.86-0.96) (p=0.0001)	0.89 (0.86-0.94) (p=0.008)	0.91 (0.86-0.96) (p=0.003)
Z-score (Median (IQR))	0.24 (-0.44-0.80)	-0.61 (-1.10-0.39)	-0.74 (-1.10-0.02)	-0.45 (-1.12-0.40)
N	(267)	(46)	(14)	(32)

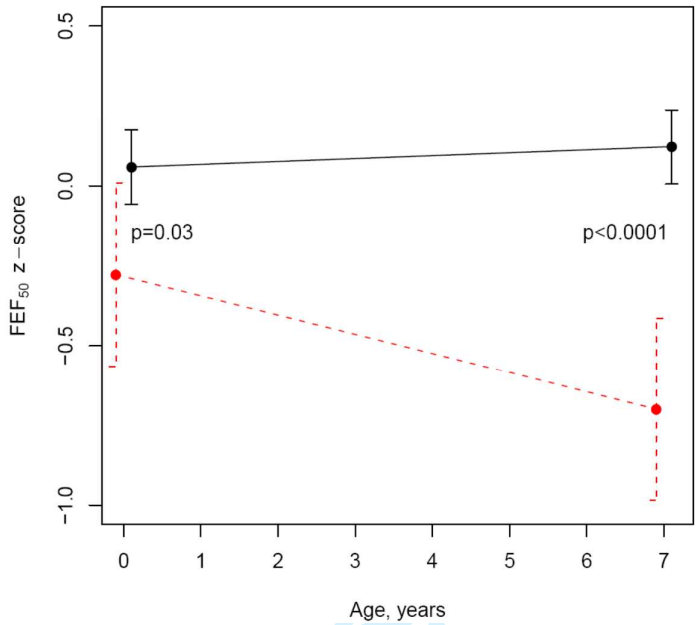
p-values in brackets correspond to test for comparing with the group "No asthma"

Table 4. Multivariable model of airflow at 7 years. For continuous measures (*) the estimate was calculated per inter-quartile range increase in covariate level. Airflow at 7 years was adjusted for height and gender.

	Child-FEF_{50} (N=270) Δ Z-score		
	Estimate	95% conf. interval	Significance
Neonatal-FEF_{50}*	0.20	[0.06;0.34]	**p=0.006**
Asthma by age 7	-0.69	[-1.01;-0.37]	**p<0.0001**
ETS at age 3*	-0.02	[-0.03;-0.00]	**p=0.01**
	Child-FEV_1 (N=275) Δ Z-score		
	Estimate	95% conf. interval	Significance
Neonatal-$FEV_{0.5}$*	0.12	[-0.02;0.27]	p=0.09
Asthma by age 7	-0.62	[-0.94;-0.29]	**p=0.0002**
ETS at age 3*	-0.01	[-0.02;0.01]	p=0.25

Figure 1

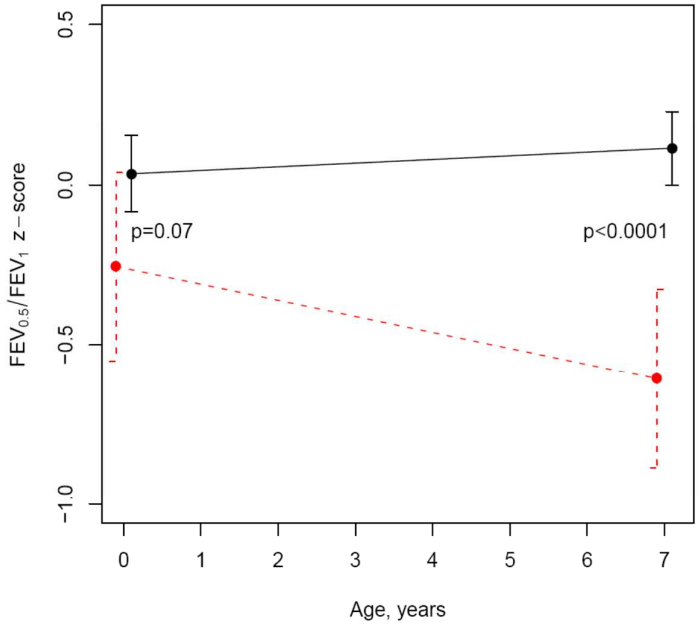
1A: Neonatal-FEF_{50} – Child-FEF_{50}



Controls

Asthma

1B: Neonatal $FEV_{0.5}$ - Child-FEV_1



Controls

Asthma

Supplemental Appendix

Interaction between Asthma and Lung Function Growth in Early Life

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&

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Methods

Data management

History was collected on-line during visits to the COPSAC clinical research unit. Objective assessments were double checked against source data and the database subsequently locked. The database was monitored by an audit trail.

Spirometry in neonates

Neonatal spirometry was obtained by forced flow-volume measurements applying the raised volume rapid thoracic compression technique. The subjects were required not to have had symptoms of respiratory infection preceding the test. The forced expiratory volume at 0.5 second, $FEV_{0.5}$(neonate), and the forced expiratory flow at 50% of vital capacity, FEF_{50}(neonate), were estimated as previously described. [1-3] Baseline was defined as the measurements after inhalation of saline.

Bronchial responsiveness to methacholine in neonates

Methacholine chloride aerosol was administered with a dosimeter attached to a nebulizer and inhaled from a metal spacer. Methacholine was given in quadrupling dose steps from 0.04 to 16.67 µmol. $PTcO_2$ was measured continuously during the methacholine challenge by the TCM3™ from Radiometer, Copenhagen, Denmark. Lung functions were repeated in duplicates after each dose until $FEV_{0.5}$ fell by at least 20% or the maximum dose was reached. One mg terbutaline was administered from a pressurized metered dose inhaler via spacer at the end of the test.

Our sensitivity analyses showed neonatal-$PD_{15}(TcO_2)$ to be more sensitive than PD determined from spirometric indices for assessment of bronchial responsiveness. We used a nonlinear dose-response model to determine the provocative dose estimates, which efficiently uses the data and provides sounder dose estimates than the traditional linear interpolation of the dose-response relationship, which is more prone to variations.[1]

Spirometry by age 7

Lung function was measured by spirometry in the child's 7th year of life using a pneumotachograph Masterscope Pneumoscreen, system 754916 spirometer (Erich Jäeger, Würtzburg, Germany). The subjects were tested sitting, and wearing nose clips. Spirometry

was assessed from up to five technically acceptable maneuvers to obtain two flow-volume curves with less than 0.2-L difference between the largest using the higher of the two as the outcome. Compliance was assured by computer-animated volume driven incentive well-known to the children and all children had trained the procedures on repeated occasions before the study day.

FeNO Measurements

Baseline FeNO was measured at 7 years of age by an online technique[4] using NIOX® FLEX (Aerocrine, Solna, Sweden) in accordance with international recognized guidelines[5]. The child was comfortably seated and breathing quietly for about 5 minutes to acclimatize. Thereafter, the child inhaled to near total lung capacity and immediately exhaled at a constant flow of 50 ml/s until a FeNO plateau of ≥2 seconds could be identified. An exhalation lasted at least 4 seconds and the expiratory pressure was maintained at 5–20 cm H_2O to close the velum. During exhalation, the child was guided by an exhalation flow driven animated computer program. Two repeated measurements that agreed within 5% were completed and mean FeNO was recorded.

Treatment Algorithm

Medical treatment for asthma followed strict predefined algorithms. For symptom relief, parents were provided with terbutaline in a pressurized metered dose inhaler (pMDI) with a spacer to be administered as needed. Recurrent asthmatic episodes as classified above defined the threshold for a 3-month course of 400µg of inhaled budesonide administered by a pMDI with a spacer. Relapsing symptoms was given as six and subsequently twelve month treatment in children responding to such treatment. Montelukast 4mg daily (5 mg after age 5) was added to the treatment of children with recurrent episodes despite budesonide maintenance treatment. Terbutaline as needed was substituted by Formoterol as needed for the treatment of children with recurrent episodes despite budesonide and montelukast maintenance treatment. Acute severe asthmatic exacerbation was treated with oral prednisolone 1-2mg/kg per day for three days (alternatively budesonide 1600µg per day for two weeks). No other treatment was allowed for asthmatic symptoms.

All subjects participated in the randomized controlled clinical trial of intermittent treatment with inhaled budesonide versus placebo for two weeks during every episode in the first three years of life, showing no short-term or long-term treatment effect.[6]

Diary registration of asthmatic symptoms

Asthmatic symptoms were recorded by the parents in daily diaries from 1 month until 6 years of age. Asthmatic symptoms were explained to the parents as wheeze or whistling sounds, breathlessness or recurrent troublesome cough severely affecting the wellbeing of the infant. Emphasis was given to symptoms from the lower airways that were not just audible but actually affecting the sleep, the activity or the well-being of the child. The symptoms were detailed in a book that was given to the parents and presented on the COPSAC website. The doctors at the research unit reviewed symptom definition and the diary entries with the parents at the 6-monthly clinical sessions during 6 years. Daily symptoms were recorded as composite dichotomized scores (yes/no) on each day.

Temporal categories of asthmatic symptoms

We calculated an individual index of asthmatic symptom load based on the available diary recordings as the ratio between the individual average and the cohort population average for a given age (days) using a novel algorithm explained below. The index is standardized for age rather than date because the cohort covers 3 years age-span per calendar year (recruited 1998-2001), and because of the strong age-dependency of symptoms.

We calculated the individual average symptom load index as the sum of days with symptoms during each of the age intervals studied (3-year, 2-year and 1-year intervals respectively). Categorization based on 2-year intervals (0-2 year, 2-4 year and 4-6 year) results in 8 possible categories of temporal asthmatic pattern (Table 2-On-Line). The expected frequencies in these 8 categories were calculated as the product of the observed frequency for each age-group assuming independency between these age-groups. These expected frequencies were compared with the observed frequencies.

Categorization based on 3-year periods (0-3 years and 3-6 years of age) yielded 17% persistent wheezers (high-high symptom load), 18% transient early wheezers (high-low symptom load), 11% late onset wheezers (low-high symptom load), and 54% asymptomatic

children (low-low symptom load). Persistent or late onset wheezers categorized 38 of 39 children with asthma by age 6 (sensitivity for current asthma by age 6: 0.97 (0.84-1.00)) while 46 of 266 non-asthmatics were categorized as persistent or late onset wheezers (specificity for current asthma by age 6: 0.83 (0.78-0.87))

Refining the age intervals to 2-year periods resulted in 8 categorical patterns. Symptom patterns between age-groups were significantly inter-dependent (chi square p-value<0.0001). Patterns of low-high-low (3%) and high-low-high (1%) were more rare than expected (14% and 6% respectively) (one-sided p-value<0.0001). By ignoring these two subsets we could confirm a general pattern similar to the classical 4 categories: "Persistent wheeze" (high-high-high) was more commonly observed than expected and constituted 12% of our birth cohort. Likewise "healthy" children (low-low-low) were more common than expected and classified 51% of the children. "Transient early" (high-low-low and high-high-low) and "late onset" (low-low-high and low-high-high) classified 23% and 14% of children which was less common than expected. There was a high level of agreement between this 2 year classification and the 3 year classification (simple kappa coefficient =0.84 (0.79-0.89)).The sensitivity of this categorization of persistent and late onset in relation to 6 year current asthma was 0.95 (0.81-0.99) and the specificity was 0.85 (0.80-0.89). This was very similar to the 3-year categorization. We therefore choose the 3-year categorization for future analyses because it is always possible to classify a child by this method.

When further reducing the intervals to 1-year symptom periods, 96 children could not be classified in the 4 categories described above and 40 children changed category. The 96 children that could not be classified exhibited what graphically seemed a random pattern from which no new archetype was apparent. This inverse relation between finer age grouping and number of children classified into archetypes is mainly induced by instabilities in the index of wheeze load as age grouping narrows.[7]

RESULTS

Baseline neonatal- $FEV_{0.5}$ and -FEF_{50} after inhalation of saline could be calculated in 403 and 398 neonates respectively.

Paired assessments of neonatal-FEF_{50} and child-FEF_{50} were available in 306 (74%) children and paired assessments of neonatal-$FEV_{0.5}$ and child-FEV_1 in 313 (76%).

Table 1-online. Multivariate model of airflow at 7 years including different measures of atopy. For continuous measures (*) the estimate was calculated per inter-quartile range increase in covariate level. Airflow at 7 years was adjusted for height and gender.

	Neonatal-FEF_{50} *vs.* Child-FEF_{50} (N=270)			**Neonatal-$FEV_{0.5}$ *vs.* Child-FEV_1 (N=275)**		
	Estimate	95% conf. interval	p-value	Estimate	95% conf. interval	p-value
Neonatal lung function	0.21	[0.07;0.36]	**0.004**	0.12	[-0.02;0.23]	0.09
Covariates:						
Inhalant sensitization 6, 18 months	0.46	[0.02;0.90]	**0.04**	0.23	[-0.22;0.68]	0.31
ETS at age 3	-0.02	[-0.03;-0.00]	**0.01**	-0.01	[-0.02;0.01]	0.25
Asthma	-0.71	[-1.03;-0.39]	**<.0001**	-0.63	[-0.95;-0.30]	**0.0002**

	Neonatal-FEF_{50} *vs.* Child-FEF_{50} (N=260)			**Neonatal-$FEV_{0.5}$ *vs.* Child-FEV_1 (N=265)**		
	Estimate	95% conf. interval	p-value	Estimate	95% conf. interval	p-value
Neonatal lung function	0.20	[0.05;0.34]	**0.008**	0.13	(-0.02;0.27]	0.08
Covariates:						
Inhalant sensitization 4 yrs	0.25	[-0.08;0.58]	0.14	0.22	[-0.12;0.56]	0.19
ETS at age 3	-0.02	[-0.03;-0.00]	**0.01**	-0.01	[-0.02;0.01]	0.29
Asthma	-0.71	[-1.03;-0.38]	**<.0001**	-0.63	[-0.96;-0.30]	**0.0002**

	Neonatal-FEF_{50} *vs.* Child-FEF_{50} (N=257)			**Neonatal-$FEV_{0.5}$ *vs.* Child-FEV_1 (N=262)**		
	Estimate	95% conf. interval	p-value	Estimate	95% conf. interval	p-value
Neonatal lung function	0.23	[0.09;0.38]	**0.001**	0.15	[0.00;0.29]	**0.04**
Covariates:						
Inhalant sensitization 6 yrs	0.03	[-0.25;0.31]	0.83	0.08	[-0.21;0.37]	0.59
ETS at age 3	-0.02	[-0.03;-0.00]	**0.01**	-0.01	[-0.02;0.01]	0.25
Asthma	-0.63	[-0.94;-0.32]	**<.0001**	-0.57	[-0.89;-0.25]	**0.0005**

	Neonatal-FEF_{50} *vs.* Child-FEF_{50} (N=233)			**Neonatal-$FEV_{0.5}$ *vs.* Child-FEV_1 (N=237)**		
	Estimate	95% conf. interval	p-value	Estimate	95% conf. interval	p-value
Neonatal lung function	0.25	[0.10;0.39]	**0.0009**	0.14	[-0.01;0.29]	0.08
Covariates:						
Eosinophils[1]	0.12	[-0.11;0.34]	0.31	0.10	[-0.14;0.35]	0.41
ETS at age 3	-0.02	[-0.03;-0.01]	0.006	-0.01	[-0.02;0.01]	0.23
Asthma	-0.77	[-1.09;-0.45]	<.0001	-0.60	[-0.95;-0.25]	0.0009
	Neonatal-FEF_{50} *vs.* Child-FEF_{50} (N=263)			Neonatal-$FEV_{0.5}$ *vs.* Child-FEV_1 (N=268)		
	Estimate	95% conf. interval	p-value	Estimate	95% conf. interval	p-value
Neonatal lung function	0.18	[0.04;0.32]	0.01	0.12	[-0.03;0.26]	0.11
Covariates:						
Atopic dermatitis	0.14	[-0.09;0.36]	0.22	0.02	[-0.21;0.25]	0.84
ETS at age 3	-0.02	[-0.03;-0.00]	0.02	-0.01	[-0.02;0.01]	0.27

Asthma	-0.73	[-1.05;-0.41]	<.0001	-0.62	[-0.94;-0.30]	0.0002

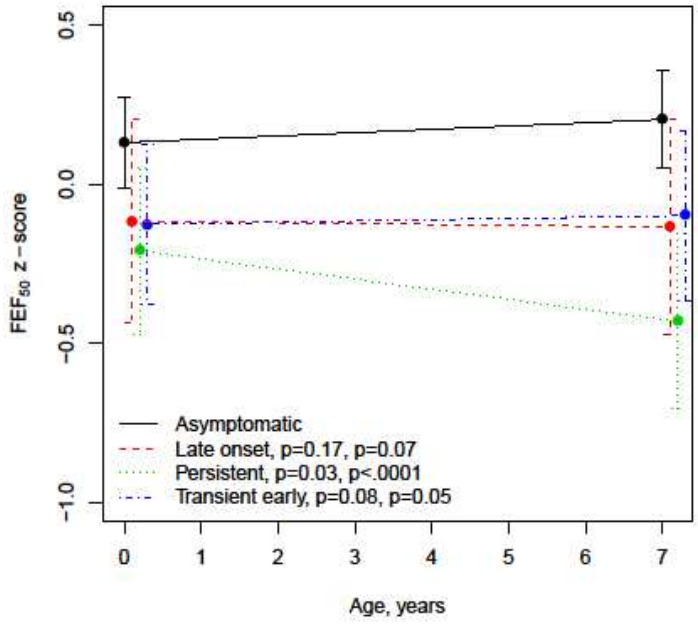
[1] (dichotomized above or below median value, $0.35*10^9$ cellls)

Table 2-online. Multivariate model of wheeze patterns and airflow at 7 years. For continuous measures (*) the estimate was calculated per inter-quartile range increase in covariate level. Airflow at 7 years was adjusted for height and gender.

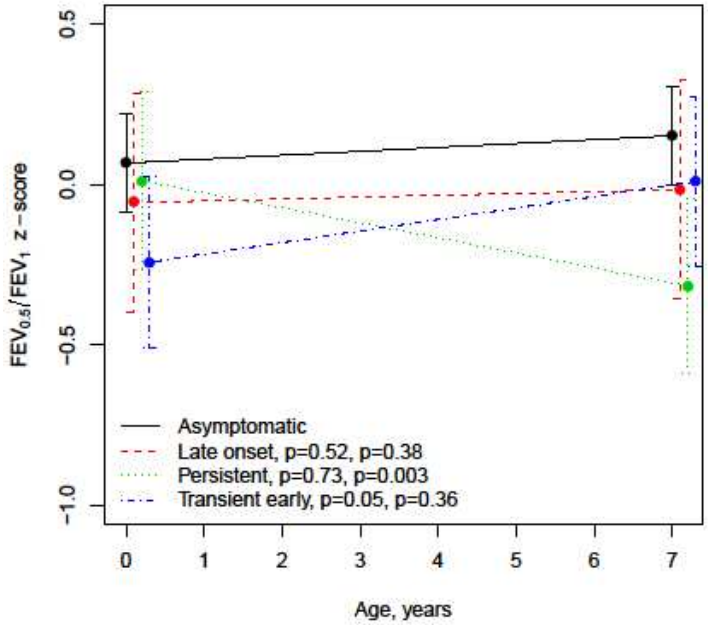
	Child-FEF_{50} (N=270) Δ Z-score		
	Estimate	95% conf. interval	P-value
Neonatal-FEF_{50}*	0.16	[0.00;0.31]	**<0.05**
Persistent wheeze	-0.65	[-0.98;-0.33]	**0.0001**
Transient early wheeze	-0.27	[-0.58;0.04]	0.09
Late onset wheeze	-0.28	[-0.66;0.10]	0.15
ETS at age 3*	-0.02	[-0.03;-0.00]	**0.02**
	Child-FEV_1 (N=275) Δ Z-score		
	Estimate	95% conf. interval	P-value
Neonatal-$FEV_{0.5}$*	0.15	[-0.00;0.30]	0.05
Persistent wheeze	-0.48	[-0.81;-0.15]	**0.004**
Transient early wheeze	-0.07	[-0.39;0.26]	0.69
Late onset wheeze	-0.08	[-0.47;0.32]	0.70
ETS at age 3*	-0.01	[-0.02;0.01]	0.32

Figure 1-online: Forced expiratory flow rate at 1 month and 7 years of age in children with different temporal categories of asthmatic symptoms from 0 to 6 years of age. Mean Z-score and 95% CI. P-values for each wheeze pattern vs asymptomatic is given (1 month, 7 years)

1A: Neonatal-FEF_{50} - FEF_{50}(child)



1B: Neonatal $FEV_{0.5}$ - Child-FEV_1



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