Paediatric lung function testing

Determinants and reference values

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Contents

CHAPTER 1

Introduction

In the last decades there has been a rising interest in paediatric lung function testing. Specific guidelines for lung function testing in children were developed and specific paediatric lung function tests became available for research and clinical use. Lung function tests are used as substitutes of lung growth and provide objective measures in diagnosis and follow-up of lung diseases.

Lung growth and development

Prenatal lung development is divided into five stages. During the *embryonic stage* (0-7 weeks) the trachea, the two main bronchi and lobar and segmental bronchi develop. In the subsequent *pseudoglandular stage* (7-17 weeks) branching of the conducting airways continues and the pulmonary circulation develops in parallel. The following stage is the *canalicular stage* (17-27 weeks) which is characterized by the formation and differentiation of the acini including primitive alveoli. During the *saccular stage* (28-36 weeks) the first real alveoli develop and the area of gas exchange increases. Alveolarization continues during the *alveolar stage* that starts at 36 weeks of gestational age.
The most rapid postnatal alveolarization takes place during the first two years of life. Alveolarization may not be complete until 8 years of age and recent findings showed that regeneration of alveoli throughout life might be possible[1]. At birth, branching is complete and the conducting airways increase in length and diameter parallel to child growth. The increase in airway diameters results in a marked fall in airway resistance. During puberty, lung growth lags behind the phase of rapid increase in height, resulting in a period of relatively low lung function for height[2]. At the age of 18-20 years, peak lung function is achieved and a plateau in lung function is observed, followed by a decline in lung function during adulthood.

Prenatal and postnatal determinants of lung growth

During the prenatal and postnatal periods both genetic and environmental factors can importantly influence development of airways and lungs. There is increasing evidence that lung function 'tracks' from birth into infancy [3,4] and childhood [5-7] and from childhood into adulthood[8-11]. This means that, in the undisturbed situation, from the early beginning lung growth continues along a certain percentile line and therefore neonatal lung function is a major determinant of adult lung function. Since prenatal and early postnatal life are the most critical periods in terms of lung growth and

development, the respiratory system is most vulnerable to harmful exposures in these periods. In this thesis the following determinants of lung growth are examined:

Genetic influences

Studies in families with asthma and COPD showed a strong correlation of spirometric measures between family members, suggesting that the variation in lung function can partly be ascribed to familial factors[12-15]. There is conflicting evidence as to what extent this familial aggregation of lung function is caused by shared genetic or by shared environmental factors. A twin-study showed that the correlation between lung function of monozygotic twins was twice the magnitude of the correlation between lung function of dizygotic twins[16]. This suggests that a large part of the found similarities in lung function are caused by genetic factors rather than body size aggregation or shared environment. Whether parental lung function is associated with infant lung function has never been studied.

Body growth

Since body growth results in growth of the respiratory system, body growth is the most important determinant of lung function. Impaired growth in utero results in decreased neonatal lung function[17,18]. In 1989, Barker et al demonstrated a relationship between accelerated infant growth and cardiovascular diseases in adults[19]. Recent studies showed that rapid growth in early postnatal life is also associated with more respiratory complaints in infancy and childhood[20,21] as well as decreased infant lung function[22,23]. However, the influence of early postnatal weight gain on lung function in later childhood was never investigated.

Smoke exposure

The most frequently studied prenatal environmental determinant of lung growth is active maternal smoking during pregnancy. This has been shown to result in impaired lung function in offspring [24]. Although studies investigating the effect of passive maternal smoking on birth weight are numerous and showed that passive maternal smoking is a significant source of foetal exposure[25], no studies investigated the effect of maternal exposure to environmental tobacco smoke (ETS) during pregnancy on infant lung function.

A meta-analysis of studies measuring lung function in childhood found decreased parameters of peripheral airway function in children whose mothers smoked during pregnancy[26]. However, these were all cross-sectional studies, lacking the possibility to study the change in lung function from infancy into childhood after prenatal smoke exposure. Only one study performed follow-up lung function measurements in children

prenatally exposed to tobacco smoke and demonstrated a waning effect of maternal smoking during pregnancy on infant lung function[27,28].
Not only prenatal but also postnatal ETS exposure was associated with decreased peripheral airway function in infancy[29], school age[30], and adolescence[31] although the effect seemed less than the effect of prenatal tobacco smoke exposure[26].
Although there is significant evidence of lung function tracking, suggesting that lung damage during early life persists into later childhood and adulthood, recent studies showed possibilities of catch-up growth of lung function during and beyond the period of postnatal maturation[32]. Therefore, one might suggest that the adverse effects of tobacco smoke exposure in utero do not persist into childhood, but that lung function in childhood is less impaired than infant lung function, as a result of catch-up lung growth in the first years of life.

Paediatric lung function measurements

Genetic and environmental determinants can importantly influence lung function parameters in the normal population over time. Furthermore, lung function tests are nowadays feasible in younger children. These changes can importantly influence reference values.

Reference values

Reference values of lung function in the normal population are required for correct interpretation of lung function in the individual child. In order to assess an individual's lung function it should be compared with the lung function of a healthy individual of the same height, age and sex. A healthy reference population is necessary to generate reference values for lung function measures. The reference population should be a representative sample of the population and the included children should be free of respiratory diseases or other diseases influencing lung function, such as neuromuscular diseases, and should have 'no more than incidental smoking experience', according to the recommendations of the GAP conference [33].
When generating reference equations for lung function measures, one should incorporate appropriate determinants, such as height, age and sex. Since the variability of lung function measures is often not constant over different ages and lung function data are generally non-normally distributed, the statistical analysis should be able to account for the variability and the skewness of the distribution in order to provide valid lower limits of normal. Modelling the variability and skewness makes it possible to provide precise estimates of an individual's z-score, expressing an

individual's position in relation to people with the same height, age and sex, taking the variation of the population into account.

Due to ongoing genetic and environmental influences on lung function, reference values should be regularly updated. Secular trends in the age-height relationship have been described[34], resulting in secular trends in lung function. Also unknown environmental factors might change over time resulting in secular change. Besides this, technical changes in measurement protocols, devices and statistical techniques might influence reference data sets. Furthermore, new lung functions tests have become available over time enabling measurements in younger children. Therefore, new reference values should be generated to prevent overdiagnosis and underdiagnosis of pulmonary diseases in the current paediatric population.

Specific and adjusted tests for infants and young children

Increasing evidence that respiratory diseases have their origin in early life has led to a revival of interest in lung function testing in infants and preschooler. Adult lung function equipment and measurement protocols have been accustomed for use in children and new tests have been developed to measure lung function in infants and preschoolers. The interrupter technique[35-38], measuring the interrupter resistance (R_{int}), requires minimal collaboration and can be used as a measure of bronchial obstruction[39]. Although studies demonstrated on average an increase in R_{int} during bronchoprovocation tests[40,41], the ability of R_{int} to detect methacholine induced bronchoconstriction in individual patients needs to be studied.

Aims and outline of the thesis

The aim of this thesis was to investigate the influence of genetic and environmental factors on lung function in healthy children at different ages and to provide new reference values for the most commonly used lung function tests throughout childhood and adolescence. Additionally, we evaluated the use of two specific lung function tests for young children.

Outline of the thesis

A healthy birth cohort is used to examine the genetic influence on neonatal lung function by determining the association between parental lung function and lung function in their offspring in **chapter two**.

In **chapter three** the influence of excessive growth in the first three months of life on wheezing and lung function in early childhood is investigated.
To explore whether the loss of lung function caused by prenatal smoke exposure persists or wanes throughout early childhood, the association between prenatal and postnatal smoke exposure on the change in lung function is studied in **chapter four**.
Since prenatal exposure to tobacco smoke results in decreased neonatal lung function, the consequences of smoke-free legislation on changes in neonatal lung function in a healthy birth cohort are investigated in **chapter five**.
In **chapter six** new paediatric reference values for the most commonly used lung function tests are presented and the influence of smoke exposure on spirometry is shown in the healthy reference population.
Since R_{int} is advocated as an alternative measure of airway obstruction, the sensitivity and specificity of R_{int} to detect bronchoconstriction during methacholine challenge testing is evaluated in **chapter seven**.
Because the success rate of exhaled Nitric Oxide measurements in young children can possibly be improved when the exhalation time is abbreviated, the agreement between six and ten seconds exhalation time is evaluated in **chapter eight**.
The main findings and implications for further research are discussed in **chapter nine**, followed by a summary in Dutch in **chapter ten**.

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CHAPTER 2

Relation between parental lung function and their offspring's lung function early in life

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Abstract

Objective:
To investigate the relation between parental lung function and their offspring's lung function measured early in life.

Methods:
Infants were participants of the Wheezing Illnesses Study Leidsche Rijn (WHISTLER). Lung function was measured before the age of 2 months using the single occlusion technique. Parental data on lung function (spirometry), medical history and environmental factors were obtained from the linked database of the Utrecht Health Project.

Results:
In 546 infants parental data on pulmonary function and major covariates were available. Univariate linear regression analysis demonstrated a significant positive relation between the infant's respiratory compliance (C_{rs}) and parental $FEF_{25-75,}$ FEV_1 and FVC. A negative significant relation was found between the infant's respiratory resistance (R_{rs}) and parental FEF_{25-75} and FEV_1. No significant relation was found between the infant's respiratory time constant (τ_{rs}) and parental lung function. Adjusting for body size reduced the significance of the observed relations, adjusting for shared environmental factors did not change the observed results.

Conclusion:
Parental lung function levels are predictors of respiratory mechanics of their newborn infants, which only partially could be explained by familial aggregation of body size. This suggests genetic mechanism in familial aggregation of lung function, which are already detectable early in life.

Introduction

A few studies have demonstrated that parameters of lung function measured early in life are predictive for respiratory symptoms and outcome early in childhood [1]. In addition, there are many data showing a genetic trait in wheezing illnesses in childhood with a dominant maternal influence [2;3], but it is not known whether "familial small airways" play a role in the inheritance of wheezing illnesses. Investigations in diverse populations have demonstrated familial aggregation of lung function at older ages [4-6], but whether the similarities of various pulmonary function testing variables are related to common familial environmental exposures or shared genes remains unclear. Several studies have shown a lack of major genetic effects on forced expiratory volume in one second (FEV_1) in general populations [7-9], whereas others suggest important genetic effects [10-12]. Moreover, Chen et al illustrated that different pulmonary function indices may have different mechanisms underlying the familial aggregation, e.g., the familial aggregation for FEV_1 is most likely controlled by multiple loci with no major gene effect and caused by shared environmental factors whereas for forced vital capacity (FVC) major genetic mechanisms are suggested [7;13].
Whether parental lung function levels are related to early life lung function in their offspring has not been reported, nor which other factors like the age, body size and medical history of parents (asthma or allergy) as well as shared environmental factors during pregnancy and shortly after birth play a role in such relation. In addition, it would be interesting to investigate whether there is a dominant maternal or paternal influence for early life lung function of offspring.
The aim of this study was to investigate in the Wheezing Illnesses Study Leidsche Rijn (WHISTLER) whether parental lung function is related to early life lung function of their offspring and which other factors like the age, body size and medical history of parents as well as shared environmental factors during pregnancy and shortly after birth play a role in this relation.

Methods

Study population

All infants in the current study are participants of the Wheezing Illnesses Study Leidsche Rijn (WHISTLER), a prospective population-based birth cohort study on determinants (including early life lung function) of wheezing illnesses. Study design and rationale of WHISTLER were described in detail elsewhere [15]. Briefly, healthy infants born in Leidsche Rijn, a new residential area under construction near the city of Utrecht, were invited by telephone to participate in this study before the age

of 2 months. Exclusion criteria were gestational age < 36 weeks, major congenital abnormalities and neonatal respiratory disease. A questionnaire filled in by one of the parents was used to gather information on gestational age, birth weight and length and exposure to tobacco smoke (active and passive maternal smoking during pregnancy and passive smoking of the child after birth). Lung function, weight and length were measured at inclusion. The paediatric medical ethics committee of the University Medical Center Utrecht approved the study. Written informed consent was obtained from the parents.

Parental data on medical history, lung function, anthropometrics and environmental factors (smoking status, exposure to pets, socio-economic status) were obtained from the linked database of the Utrecht Health Project (Dutch acronym LRGP: Leidsche Rijn Gezondheids Project), a large health monitoring study in Leidsche Rijn, which aims to generate data from all inhabitants on determinants of health and disease as described previously [15;16]. The medical ethics committee of the University Medical Center Utrecht approved the study. Written informed consent was obtained from all participants.

Lung function tests

Infant lung function was measured before the age of two months. Measurements were performed during natural sleep without the use of any sedation. Data collection was confined to consecutive periods of quiet sleep in which posture was stable and respiration was regular. Lung function was assessed from measurement of passive respiratory mechanics (resistance (R_{rs}), compliance (C_{rs}) and time constant (τ_{rs}) of the respiratory system) using the single occlusion technique (SOT)[17]. Airflow was measured using a heated Lilly-type pneumotachometer (series 8300, dead space 1.66 ml, resistance 0.4 cm H_2O at 5 L/min, Hans Rudolph Inc., Kansas City, MO, USA) attached to a face mask (infant mask, Hans Rudolph Inc., Kansas City, MO, USA). The mask was sealed to the infant's face using therapeutic silicone putty (Magic Putty, Oldelft Benelux BV, Delft, the Netherlands) to prevent air leaks and to minimize dead space. Pressure changes at the airway opening were measured with a pressure transducer (Honeywell, type 163PC01D75, Morristown, NJ, USA). Volume was obtained by electronic integration of the airflow signal. Flow, volume and pressure were digitized with a sampling rate of 200 Hz and interfaced to a computer for real-time display, storage and analysis. Before each measurement, calibration of flow and volume signals was performed using a 100-ml precision syringe (Viasys Health, Höchberg, Germany). The pressure transducer was calibrated over the expected range using a pressure transducer tester (VeriCal™, Utah Medical Products Inc., Utah, USA). To be considered acceptable, each occlusion was required to meet the criteria of the ERS/ ATS Task Force on Infant Lung Function[18]. At least three technically acceptable occlusions were used to calculate mean C_{rs}, R_{rs} and τ_{rs} values. Lung function data

were calculated offline using a custom-built software package (Luna 1.7, Utrecht, the Netherlands).
Parental lung function was evaluated with a Vitalograph 2120 (Vitalograph Ltd, Buckingham, UK). At least three forced expirations were performed in accordance with the guidelines of the American Thoracic Society [19]. The maximum of the three measurements was used. The lung function variables used in the analysis were: forced expiratory flow between 25% and 75% of FVC (FEF_{25-75}), forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC). The ratio of FEF_{25-75}/FVC was calculated, a relatively size-independent measure of airway calibre[20].

Definition of variables

The role of factors like the age, body size and medical history of parents as well as shared environmental factors during pregnancy and shortly after birth (smoking status of parents, exposure to pets, socio-economic status) in the relation with lung function of parents and their offspring was examined. A positive history of asthma or bronchitis was defined as parents having been diagnosed with of asthma or bronchitis in the last 12 months. A positive history of allergy included allergy to pollen, house dust mite, pets, drugs or food. Based on the questionnaire of the Utrecht Health Project, parents were divided in three smoking categories (never, ex- and current smoker). Based on the WHISTLER questionnaire, three additional smoking variables were available (active and passive maternal smoking during pregnancy and passive smoking of the child after birth). Socio-economic status was based on educational level and defined as low (no formal education, lower secondary education or intermediate secondary education), middle (higher secondary education) or high (higher vocational or university education). The ethnic origin was classified as Caucasian versus non-Caucasian.

Statistical analysis

Prior to modeling, all variables were checked for normality of distribution and when necessary logarithmic transformations were applied. Z-scores for parental lung function variables and height and weight were calculated. Linear regression analysis was used to examine the relation between parental lung function variables (sum of absolute values of paternal and maternal forced expiratory flow between 25% and 75% of FVC (FEF_{25-75}), forced expiratory volume in one second (FEV_1),forced vital capacity (FVC) and sum of the ratio of FEF_{25-75}/FVC) and their offspring's respiratory resistance (R_{rs}), compliance (C_{rs}) and time constant (τ_{rs}). Univariate regression models were constructed with lung function variables of the offspring as dependent (outcome) variables and the sum of maternal and paternal lung function variables as the independent variables (model I). Subsequently, five multiple linear regression

models were constructed to investigate the influence of respectively age, gestational age and sex (model II), body size of the infant at the time of visit for lung function measurement (model III) and at birth (model IV), body size of parents (model V) as well as exposure to pets, parental socio-economic status (SES), parental smoking status, and parental asthma and allergy status (model VI). Analysis were repeated for maternal and paternal lung function variables separately. Normality of residuals distribution was checked to assess the fit of the models. Results are presented as linear regression coefficients and 95% confidence intervals. Intervals not including zero (p-value ≤ 0.05) were considered statistically significant. Statistical analysis was performed using SPSS Windows, version 15.0, 2001, Chicago, USA.

Results

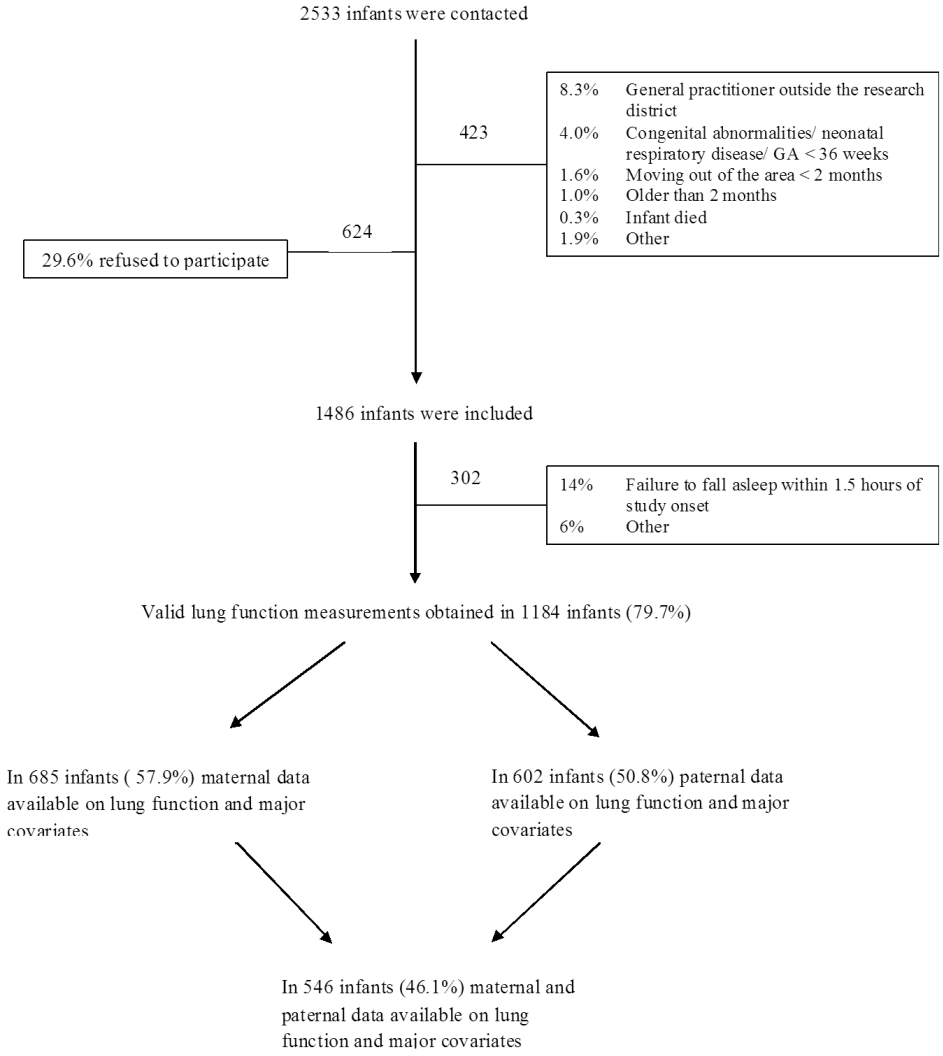
Demographic and clinical characteristics of parents and offspring

Figure 1 shows an overview of recruitment and inclusion of infants in the WHISTLER-study. Among the 1486 included infants, valid lung function measurements were obtained in 1184 infants (79.7%). Failure to obtain technically acceptable measurements was mainly due to failure to fall asleep naturally within 1.5 hours of study onset (14%). Of the infants with successful lung function, maternal data on pulmonary function and major covariates could be derived from the linked database of the Utrecht Health Project in 685 (57.9%) cases (352 female infants) and paternal data in 602 (50.8%) cases (313 female infants). In 546 infants both maternal and paternal data on pulmonary function and major covariates were available. The mean and standard deviations of age, height, weight, levels of lung function, and the frequency distribution of educational level, smoking status, exposure to pets and allergy and asthma status of the parents and their newborn infant are shown in tables 1 and 2 respectively. Fathers had significantly larger values for all lung function variables, height and weight and there was a two year age difference between fathers and mothers. Among fathers there were more current smokers and education was lower compared to the mothers. Male offspring had a significantly higher birth weight and length and weight and length at the time of lung function measurement compared to female offspring.

Lung function of parents and offspring

Table 3 shows the results of the univariate linear regression analysis with the sum of parental lung function variables as the independent variables and their offspring's lung function variables as the dependent (outcome) variables (model I). A significant

Figure 1. Overview of the inclusion of infants.



positive relation between respiratory compliance (C_{rs}) of the infant and parental FEF_{25-75}, FEV_1 and FVC was found. A significant negative relation between respiratory resistance (R_{rs}) of the infant and parental FEF_{25-75} and FEV_1 was found. The relation between R_{rs} and FEF_{25-75}/FVC was borderline significant. No significant relation was found between the respiratory time constant (τ_{rs}) of the infant and parental lung function variables. Figures 2, 3 and 4 show the results of the multiple linear regression models. After adjusting for respectively age, gestational age and sex (model II) as well as exposure to pets, parental socio-economic status (SES), parental smoking

Table 1. Demographic and clinical characteristics of parents

Variable	Mother n=685	Father n=602
General characteristics (mean ± SD)		
Age (yrs)	30.8 ± 4.1	32.9 ± 4.4
Height (m)	169.9 ± 7.0	183.3 ± 8.3
Z-score	0 (-3.34 – 2.98)	0 (-7.21 – 2.96)
Weight (kg)	70.8 ± 12.5	84.6 ± 11.4
Z-score	0 (-2.50 – 4.32)	0 (-2.42 – 4.87)
FEF_{25-75} (l/s)	3.91 ± 0.91	4.97 ± 1.21
Z-score	0 (-2.72 – 3.89)	0 (-2.46 – 4.31)
FEV_1 (l)	3.27 ± 0.50	4.38 ± 0.69
Z-score	0 (-2.98 – 3.13)	0 (-3.11 – 3.08)
FVC (l)	3.79 ± 0.60	5.23 ± 0.83
Z-score	0 (-2.70 – 3.89)	0 (-3.49 – 2.90)
FEF_{25-75}/FVC	1.05 ± 0.25	0.96 ± 0.25
Z-score	0 (-2.40 – 3.67)	0 (-2.30 – 3.56)
Questionnaire data		
History of asthma/ bronchitis (%)	6.8	6.5
History of allergy (%)	42.9	41.5
Smoking status (%)		
Never	61.5	55.1
Ex-smoker	28.3	27.7
Current smoker	10.2	17.2
Socio-economic status (%)		
Low	4.1	4.7
Moderate	30.0	36.3
High	65.9	59.0
Ethnicity (%)		
Caucasian	81.1	83.6
Non-Caucasian	18.9	16.4
Exposure to pets (%)	43.1	43.8

* Data presented as mean and standard deviation or percentages
** Z-scores expressed as mean and range
*** FEF_{25-75} = forced expiratory flow between 25% en 75%; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity;

status, and parental asthma and allergy status (model VI) the observed relations remained statistically significant. The relation between R_{rs} and FEF_{25-75}/FVC was statistically significant in all multiple linear regression models. Adjusting for body size explained in part the relation between parental lung function and their offspring's lung function. The significance of the relation between C_{rs} and parental FVC (β=0.02,

Table 2. Demographic and clinical characteristics of male and female offspring

Variable	Female offspring n=352	Male offspring n=333
General characteristics (mean ± SD)		
Gestational age (wks)	40.0 ± 1.2	39.8 ± 1.4
Age at time of visit (wks)	4.7 ± 1.3	4.6 ± 1.2
Birth weight (gr)	3460 ± 450	3593 ± 507
Birth length (cm)	50.6 ± 2.0	51.5 ± 2.2
Weight at visit (gr)	4275 ± 544	4555 ± 660
Length at visit (cm)	54.3 ± 2.3	55.1 ± 2.8
Lung function data (mean ± SD)		
Compliance C_{rs} (ml/kPa)	44.4 ± 11.1	44.2 ± 10.9
Resistance R_{rs} (kPa/l/s)	7.0 ± 2.2	7.3 ± 2.2
Time constant τ_{rs} (s)	0.308 ± 0.116	0.319 ± 0.114
Questionnaire data		
Active maternal smoking during pregnancy (%)	5.4	4.8
Passive maternal smoking during pregnancy (%)	14.5	13.2
Passive smoking infant after birth (%)	2.3	2.4

p=0.075) and between R_{rs} and parental FEV_1 (β=-0.03, p=0.073) was reduced and only showed a trend after adjusting for body size at the time of visit for lung function measurement (model III). The relation between C_{rs} and parental FVC disappeared after adjusting for length and weight at birth (model IV, β=0.02, p=0.135). Adjusting for weight and length of the parents (model V) did not change the observed results. Table 4 shows the results of the univariate linear regression analysis with maternal and paternal lung function variables as the independent variables and their offspring's lung function variables as the dependent (outcome) variables (model I). For the mother-infant pairs, univariate linear regression analysis demonstrated a significant positive relation between C_{rs} of the infant and FEF_{25-75}, FEV_1 and FVC. Adjusting for body size and shared environmental factors (model II-V) did not change the observed relations. A significant positive relation was also found between the respiratory time constant τ_{rs} and maternal FEV_1 and FVC. The relation between τ_{rs} and maternal FEV_1 and FVC however disappeared after adjusting for length and weight at visit and at birth (model III and IV). Adjustments for age, gestational age and sex (model II), maternal weight and length (model V) and exposure to pets, maternal socio-economic status (SES), maternal smoking status, and maternal asthma and allergy status (model VI) did not change the observed results. No significant association was found between maternal

Table 3. The relation between lung function of parents and offspring: unadjusted linear regression coefficients and 95% confidence interval

	Ln C_{rs} (ml/kPa)			Ln R_{rs} (kPa/l/s)			Ln τ_{rs} (s)		
	β-coefficient (95% CI)	**R^2**	**p-value**	**β-coefficient (95% CI)**	**R^2**	**p-value**	**β-coefficient (95% CI)**	**R^2**	**p-value**
FEF_{25-75} (L/s)	0.019 (0.005- 0.032)	0.013	***0.007***	-0.022 (-0.038- -0.006)	***0.014***	***0.006***	-0.003 (-0.022 - 0.016)	0.0001	0.749
FEV_1 (L)	0.045 (0.022 - 0.068)	0.027	***<0.001***	-0.034 (-0.061- -0.011)	***0.011***	***0.013***	0.011 (-0.021 - 0.043)	0.001	0.492
FVC (L)	0.032 (0.013 - 0.051)	0.020	***0.001***	-0.012 (-0.035 - 0.010)	0.002	0.278	0.020 (-0.006 - 0.046)	0.004	0.140
FEF_{25-75}/FVC	0.013 (-0.044 - 0.070)	0.003	0.662	-0.062 (-0.128 - 0.004)	0.006	***0.064***	-0.050 (-0.127 - 0.028)	0.003	0.210

FEF_{25-75} = sum of paternal and maternal forced expiratory flow between 25% en 75%; FEV_1 = sum of paternal and maternal forced expiratory volume in 1 second; FVC = sum of paternal and maternal forced vital capacity; R_{rs}= resistance of the respiratory system; C_{rs}= compliance of the respiratory system, τ_{rs} = time constant of the respiratory system.

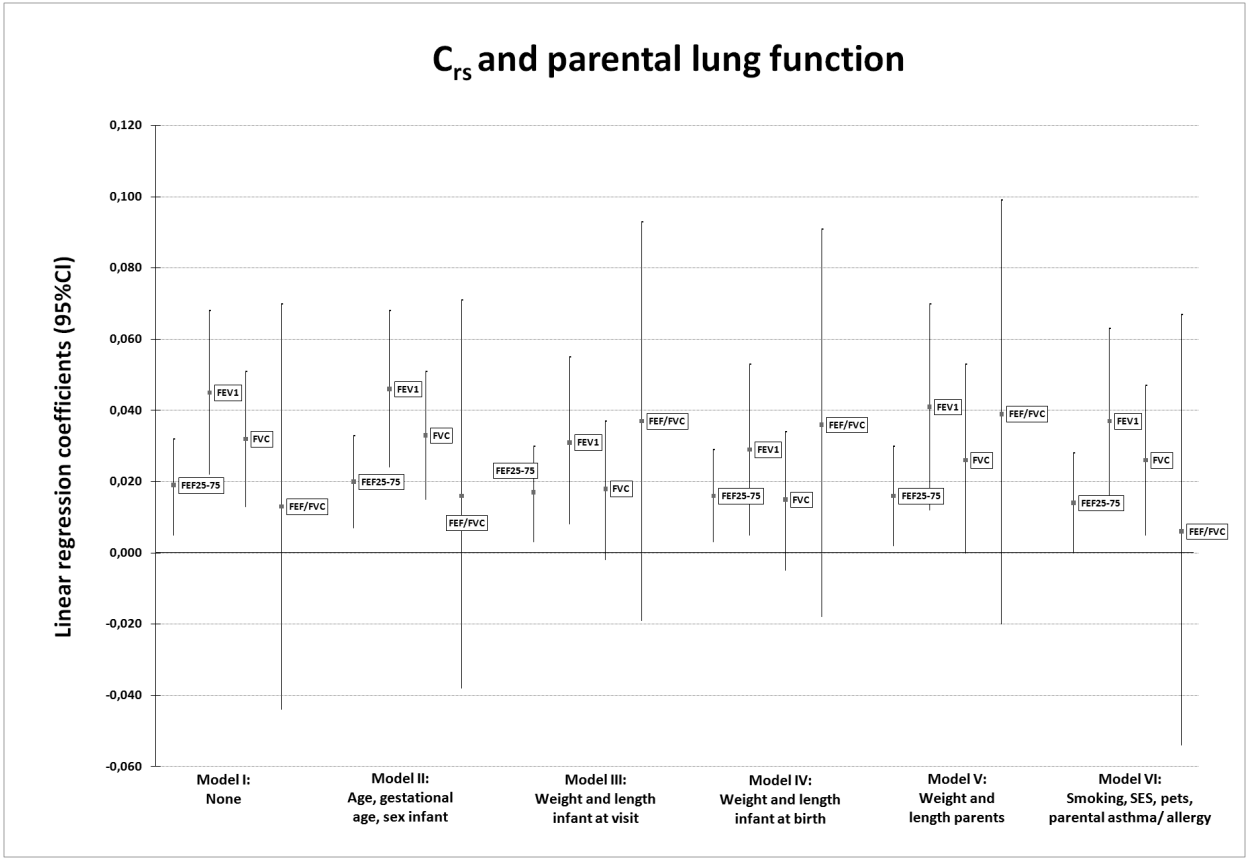


Figure 2. The relation between parental lung function (FEF_{25-75} = forced expiratory flow between 25% en 75%; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; ratio FEF_{25-75}/ FVC) and compliance (C_{rs}) of their offspring: unadjusted and adjusted linear regression coefficients and 95% confidence interval

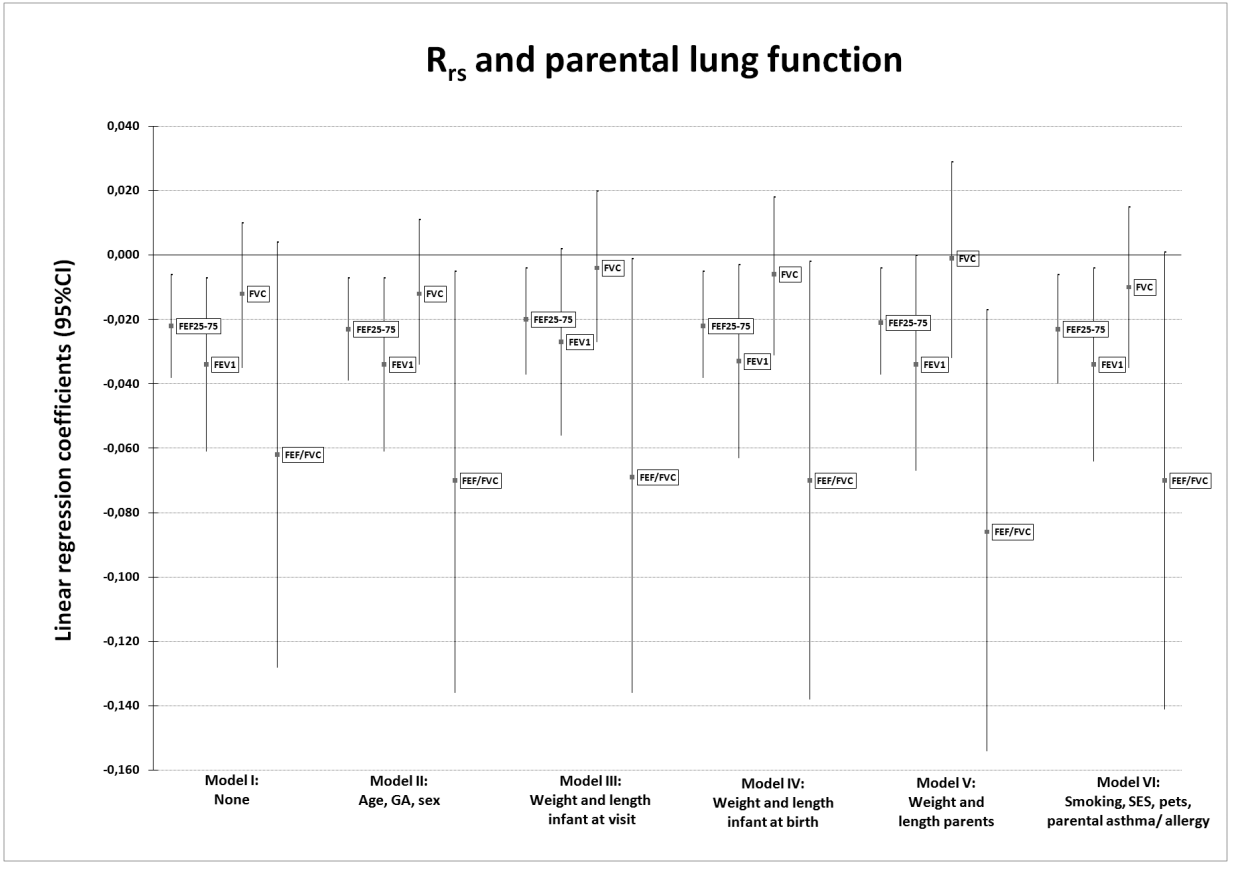


Figure 3. The relation between parental lung function (FEF_{25-75} = forced expiratory flow between 25% en 75%; FEV_1 forced expiratory volume in 1 second; FVC = forced vital capacity; ratio FEF_{25-75}/ FVC) and resistance (R_{rs}) of their offspring: unadjusted and adjusted linear regression coefficients and 95% confidence interval

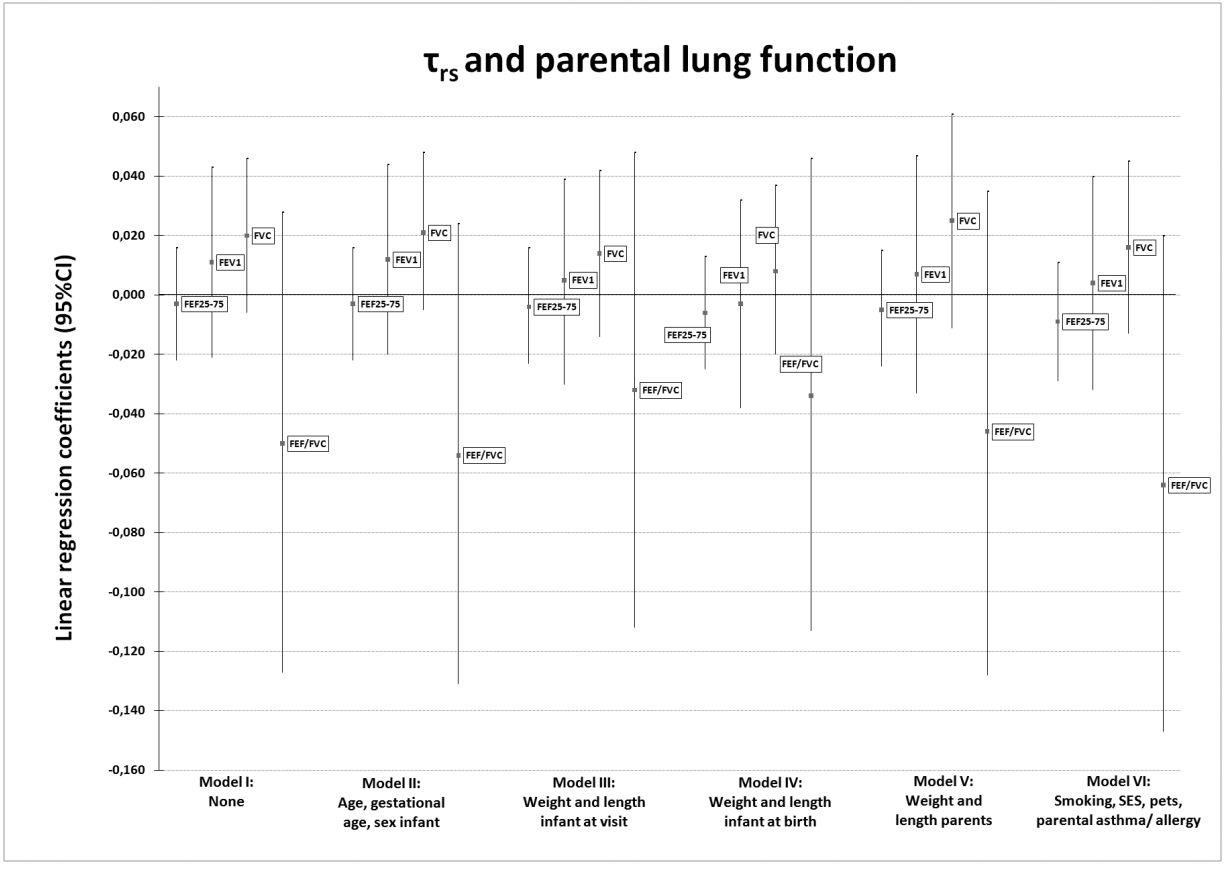


Figure 4. The relation between parental lung function (FEF_{25-75} = forced expiratory flow between 25% en 75%; FEV_1 forced expiratory volume in 1 second; FVC = forced vital capacity; ratio FEF_{25-75}/ FVC) and time constant (τ_{rs}) of their offspring: unadjusted and adjusted linear regression coefficients and 95% confidence interval

Table 4. The relation between lung function of mother, father and offspring: unadjusted linear regression coefficients and 95% confidence interval

	Ln C_{rs} (ml/kPa)			Ln R_{rs} (kPa/l/s)			Ln τ_{rs} (s)		
	β-coefficient (95% CI)	**R^2**	**p-value**	**β-coefficient (95% CI)**	**R^2**	**p-value**	**β-coefficient (95% CI)**	**R^2**	**p-value**
FEF_{25-75} (L/s)									
Mother	0.033 (0,012- 0,054)	0.014	***0.002***	-0.018 (-0,042- 0,005)	0.003	0.130	0.014 (-0,014- 0,043)	0.001	0.319
Father	0.009 (-0,008- 0,025)	0.002	0.299	-0.024 (-0,043 - -0,005)	0.010	***0.015***	-0.015 (-0,038 - 0,008)	0.003	0.191
FEV_1 (L)									
Mother	0.085 (0,047 - 0,123)	0.028	***0.001***	-0.032 (-0,075- 0,011)	0.003	0.143	0.053 (0,001 - 0,1043)	0.006	***0.045***
Father	0.026 (-0,003- 0,055)	0.005	0.075	-0.045 (-0,078 - -0,011)	0.011	***0.010***	-0.018 (-0,058 - 0,021	0.001	0.368
FVC (L)									
Mother	0.062 (0,030 - 0,093)	0.021	***0.001***	-0.009 (-0,045- 0,027)	0.0001	0.627	0.053 (0,010 - 0,096)	0.008	***0.016***
Father	0.020 (-0,004- 0,044)	0.004	0.111	-0.022 (-0,050 - 0,005)	0.004	0.114	-0.003 (-0,036 - 0,030)	0.0001	0.862
FEF_{25-75}/ FVC									
Mother	0.012 (-0.065 – 0.089)	0.0001	0.757	-0.052 (-0.139 - 0.035)	0.002	0.244	-0.040 (-0.144 - 0.065)	0.001	0.455
Father	-0.047 (-0.039 – 0.133)	0.002	0.280	-0.059 (-0.154 – 0.035)	0.003	0.215	-0.062 (-0.173 – 0.048)	0.002	0.269

FEF_{25-75} = forced expiratory flow between 25% en 75%; FEV_1 forced expiratory volume in 1 second; FVC = forced vital capacity; R_{rs}= resistance of the respiratory system; C_{rs}= compliance of the respiratory system, τ_{rs} = time constant of the respiratory system.

lung function levels and R_{rs}, except after adjusting for maternal weight and length (model V) with a borderline significant relation between R_{rs} and FEF_{25-75}/ FVC (β=-0.08, p=0.070) and after adjusting for exposure to pets, maternal socio-economic status (SES), maternal smoking status, and maternal asthma and allergy status (model VI) with a borderline significant relation between R_{rs} and FEF_{25-75} (β=-0.03, p=0.054) and FEV_1 (β=-0.04, p=0.073).
For the father-infant pair (table 4), there were no significant associations between paternal lung function variables and C_{rs}, except after adjusting for age, gestational age and sex (model II) with a significant positive relation between C_{rs} and FEV_1 (β=0.03, p=0.026) and FVC (β=0.03, p=0.033). For R_{rs}, FEF_{25-75} and FEV_1 showed a significant negative relation which did not change after adjusting for body size and shared environmental factors (model II-VI), except for the relation between R_{rs} and parental FEV_1 (β=-0.03, p=0.063) only showing a trend after adjusting for infant body size at the time of visit for lung function measurement (model III). No significant association was found between paternal lung function levels and τ_{rs}.

Discussion

In this study, we found that parental lung function is a determinant of their offspring's lung function early in life. This relation could in part be explained by familial aggregation of body size. This relation could not be explained by other factors like the age, body size and medical history of parents or shared environmental factors during pregnancy and shortly after birth. This suggests genetic mechanisms in familial aggregation of lung function, which are already detectable very early in life. To our knowledge, this is the first study investigating the influence of parental lung function parameters in the prediction of their offspring's lung function very early in life.
Some methodological aspects need to be considered. The group of infants selected for this study was a sample from all infants participating in WHISTLER. Selection was based on whether the parents participated in the Utrecht Health Project, as this study provided the parental data. Although parental data could not be compared between included and excluded infants, the baseline characteristics and lung function variables of the excluded infants were similar to those of the infants included for this study (data not shown). Therefore, it is unlikely that selective participation has affected our results. The SOT is a suitable and non-invasive method to measure lung function, but the individual assessment, especially the reliability of the measurements needs to be critically evaluated[17;21]. Difficulties in the underlying assumptions of complete relaxation, equilibration of pressures and a single time constant for the respiratory system could have influence on the validity and accuracy of measurements In

order to ensure that only technically satisfactory data were analysed and reported, measurements were performed by trained personnel according to the criteria of the ERS/ATS Task Force [18].

Although we are comparing lung function variables assessed by two different lung function techniques, it seems reasonable to assume that genetically or environmentally mediated determinants of lung function, including the size of the airways and lungs and the lung elastic recoil and resistance properties will be detected by both techniques. The inverse relation between the sum of parental FEV_1, FEF_{25-75} and FEF_{25-75}/ FVC and their offspring's R_{rs} is understandable as all parameters are a reflection of airway caliber (e.g. decrease of FEV_1 and FEF_{25-75} with higher resistance). In contrast no significant relation was found between the parental lung volume parameter FVC and R_{rs}, For the offspring's C_{rs}, a significant positive relation was found with parental FEV_1, FEF_{25-75} and FVC. C_{rs} reflects composite elastic properties of the infant total respiratory system which apparently correlates with both airway caliber and lung volume characteristics in parents. As proposed by Tager et al, FEF_{25-75}/ FVC is a measure of airway size relative to lung size ("relative airway size") and in contrast to R_{rs}, C_{rs} was not related to this variable. The time constant τ_{rs} is the time necessary for approximately 63% of the lung to empty and equal to the product of respiratory compliance and resistance. Parental lung function variables were negatively associated with R_{rs} and positively related to C_{rs}, which explains that no significant relation was found between parental lung function variables and τ_{rs}. Maternal lung function however showed a significant relation with their offsprings τ_{rs}, most likely due to the dominant maternal effect on C_{rs}.

Lung function is known to aggregate in families. A familial effect on measurements of FEF_{50}, FEF_{25-75}, FEV_1, FVC and FEF_{25-75}/ FVC at older ages has been shown [4-6;22], but there is conflicting evidence as to whether this is genetically determined or due to shared environments. In this study, we found a significant relation between several parental lung function variables and respiratory resistance and compliance of their offspring early in life. A genetic basis for the findings in our study is supported by the fact that after adjusting for shared environmental factors during pregnancy and shortly after birth, such as smoking status of the parents, exposure to pets, parental asthma and allergy status, and socio-economic status, the observed relations remained significant.

To what extent familial aggregation of lung function is primarily a reflection of familial aggregation of body size has been a source of controversy. It is generally agreed that height aggregates in families and pulmonary function measurements are dependent on height [23]. Lebowitz et al presented strong familial aggregation for FVC, FEV_1 and V_{MAX50}, but these relations disappeared after controlling for body size [24]. In contrast, Kauffmann et al found that adjustment for body size did not affect the magnitude of

the parent-child correlations for FEF_{23-75}, FEV_1, or FVC [5]. In our study, we found that familial aggregation of weight and length was in part an explanatory variable for the observed relation between parental lung function variables and lung function of their offspring.

It is interesting to note that other studies found a greater correlation in FEV_1 and other lung function variables between mothers and offspring compared to fathers and offspring [5;9]. In this study, we also found differences in the relation between maternal and paternal lung function levels and lung function level of their newborn infant. Gender of the parent modifies the relation between parental lung function and lung function of their offspring with a more dominant effect of maternal lung function on their offspring's respiratory compliance and time constant and a more dominant effect of paternal lung function on their offspring's respiratory resistance. There are some interpretations found in the literature. These include exclusive exposure to maternal genetic or environmental factors during pregnancy, differences in shared postnatal environmental exposures, hormonal differences and genetic imprinting, where the genetic factors exert their effects dependent on whether they were inherited from father or mother [25]. In addition, Holberg et al observed a significant maternal-offspring correlation in FEV_1 in asthmatic families and suggested a connection with the maternal environment in utero, more in specific that while both parents may contribute to the susceptibility of atopic disease, additional environmental effects with a maternal influence may influence the expression of the genetic factors and subsequently affect lung function[9]. In our study, a positive maternal or paternal history of asthma or allergy did not change the observed associations between parental lung function and lung function of their offspring.

In conclusion, we demonstrated as part of a large prospective population-based birth cohort study on determinants of wheezing illnesses (Wheezing Illnesses Study Leidsche Rijn or WHISTLER) that parental lung function levels are predictors of respiratory mechanics of their newborn infants, which in part could be explained by familial aggregation of body size. This suggest genetic mechanisms in familial aggregation of lung function, which are already detectable very early in life. Although currently speculative, the findings of this study may contribute to the understanding of the genetic mechanism of lung function and subsequently the development and progression of lung disease in childhood and beyond.

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CHAPTER 3

Rapid weight gain in the first months of life: a cause of early life wheezing illnesses?

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Abstract

Rationale: Rapid weight gain in infancy could be a risk factor for respiratory symptoms.

Objectives: To investigate whether rapid weight gain in the first 3 months of life is associated with more wheezing illnesses and reduced lung function in the first years of life.

Design: Prospective birth cohort.

Setting: Primary health care centres in a new residential area in the Netherlands.

Participants: Healthy newborns. Exclusion criteria were gestational age <36 weeks, major congenital abnormalities and neonatal respiratory disease. Information on first year growth and respiratory symptoms was obtained in 1431 infants, in 1335 children medical records were available and 235 children were followed up for lung function measurement at five years of age.

Main outcome measures: Wheezing symptoms between the 4th and 12th month of age from monthly questionnaires, primary care consultations for wheezing illnesses during total follow-up and forced expiratory volume in 1 second (FEV_1) and maximal mid expiratory flow (FEF_{25-75}) at five years of age.

Results: Every standard deviation higher early weight gain showed a 44% higher rate of days with wheezing symptoms in the first year (Incidence Rate Ratio: 1.44, 95% CI 1.33 to 1.57, P<0.001) and a 10% higher rate of wheezing associated primary care visits (IRR 1.11, 95% CI 1.04 to 1.19, p=0.002), after confounding adjustment. These associations were independent of birth weight. FEV_1 and FEF_{25-75} were significantly reduced with 2.8% and 5.3% with every standard deviation more weight gain.

Conclusion: Excessive weight gain in the first three months of life is a risk factor for clinically relevant wheezing illnesses in the first years of life and lower lung function at school age.

Introduction

Wheezing illnesses are highly prevalent during childhood. Almost half of all children experience wheezing during the first years of life and about 10% experiences asthma beyond the age of six[1,2]. Wheezing illnesses have a major impact on children and their families[3] and account for a large number of primary health care consultations in the first years of life[4].

The prevalence of wheezing illnesses in affluent countries has been increasing[55] parallel to the prevalence of obesity[6]. Although wheezing illnesses seem to be related to obesity, data in children are conflicting[7-9]. Rapid weight gain in the first years of life is a risk factor for the development of obesity[10,11], but also for other chronic conditions, like cardiovascular disease and type 2 diabetes[12]. A few studies suggested that rapid weight gain during infancy is also a risk factor for respiratory morbidity and decreased infant lung function. In children with frequent intermittent wheezing, rapid weight gain between birth and the age of 3 was associated with urgent physician visits and more frequent prednisone courses[13]. Accelerated weight gain during infancy was associated with more wheezing at the age of three [14], as well as in young adulthood[15]. Additionally, rapid postnatal weight gain was associated with impaired lung function development in infancy[16,17].

Importantly, none of these studies focused on weight gain in the first three months of life, while this may be a critical growth period. A recent study showed that rapid weight gain in the first 3 months of life, but not in other quarters of the first year of life, was associated with several determinants of cardiovascular disease measured in young adulthood[12]. Although the underlying mechanism of the association between rapid weight gain and cardiovascular disease may be different from that of rapid weight gain and respiratory outcomes, the first 3 months after birth do seem to be a critical growth period. Moreover, previous studies did not investigate respiratory symptoms and consultations in infancy as outcome, nor did they examine the effect of accelerated growth in the first months of life on lung function in childhood. More information on the relation between rapid early weight gain and wheezing illnesses in healthy infants is needed to support evidence-based patient information on feeding and growth of newborns and reducing the burden for families and the health care system.

In a large prospective birth cohort of healthy infants we study whether rapid growth in the first 3 months of life is associated with the number of days with wheezing symptoms in the first year of life, the number of primary care consultations for wheezing in the next years and lung function at five years of age.

Methods

Study design and study population

Infants participate in the ongoing WHeezing Illnesses STudy LEidsche Rijn (WHISTLER), a prospective birth cohort on respiratory illnesses that started December 2001[18]. Exclusion criteria are gestational age <36 weeks, major congenital abnormalities and neonatal respiratory disease. Briefly, healthy newborns were asked to participate and at the age of 3-8 weeks information on pre- and postnatal risk factors was obtained by questionnaires and lung function was measured using the single occlusion technique during natural sleep[19,20]. At the age of five children were invited for a second lung function measurement. The medical ethics committee of the University Medical Centre Utrecht approved the study. Written informed parental consent was obtained.

Determinant and outcome measurements

Birth weight and length were measured in the hospital or by the midwife in a standardized way by using a standard electronic scale and an infant stadiometer. In the Netherlands infants regularly visit Child Health Care Centres for standardized anthropometry. We asked parents to report these anthropometric measures in monthly questionnaires.

Follow-up for wheezing during the first year of life was achieved by daily questionnaires filled in by the parents. Parents were carefully instructed by one of the investigators on how to recognize wheezing. Wheezing was defined as a positive answer to the question "Did your child wheeze (whistling sound from the chest) today?" New questionnaires and (if necessary) reminders were sent monthly.

Data on primary care visits during the first years of life were obtained from the general practitioners' electronic patient files. Physician-diagnosed wheeze is assessed using different categories of wheezing illnesses in primary care, according to the International Classification of Primary Care (ICPC).

At the age of five years information about respiratory symptoms during the last years was assessed by a questionnaire and forced vital capacity (FVC) manoeuvres were obtained using a heated Lilly head pneumotachometer system (Viasys Healthcare, Hochberg, Germany). Measurements were BTPS corrected and performed conform the latest ATS/ERS statement for lung function measurements in preschoolers[21]. At least two reproducible flow-volume curves were obtained. The largest forced expiratory volume in 1 second (FEV_1) was selected and maximal mid-expiratory flow (FEF_{25-75}) was obtained from the curve with highest sum of FEV_1 and FVC.

Analysis

To assess differences between children with and without data on growth, with and without available medical records and with and without lung function measurement at five years of age, chi-square-tests and t-tests were used. Z-scores of weight were calculated at birth and at 3 months, indicating the ranks in the respective weight distributions. As not all children were weighed at exactly 3 months, the weight closest to this age was used (minimum age 60 days, maximum age 120 days) and z-scores were adjusted for the exact age in days. Weight gain was calculated as the difference between z-scores of weight at birth and at 3 months of age. Subsequently, rapid weight gain was defined as a change greater than 0.67 z-score, normal weight gain as a change in z-score between -0.67 and 0.67 and slow weight gain as a change of -0.67 z-score or less[13,22]. To assess possible confounding, baseline characteristics of groups of children with these three different weight gain patterns were tested using chi-square, ANOVA or Kruskal-Wallis tests, where appropriate.

Number of days with wheezing symptoms between the 4^{th} and 12^{th} month of age was used as a count type outcome, best fitting a negative binomial distribution, due to many children with zero days with wheezing symptoms. Negative binomial regression was used with number of days with wheezing symptoms between the 4^{th} and 12^{th} month as a dependent variable and weight gain as an independent continuous variable. The number of returned monthly questionnaires was used as an offset variable to indicate exposure time. Firstly, the crude association was calculated. Secondly, the model was adjusted for gender and gestational age. Thirdly, the model was additionally adjusted for maternal smoking during pregnancy, parental smoking after birth, duration of exclusive breastfeeding, parental allergy, siblings and ethnicity of the mother as possible confounders. To determine whether the effect was present in children with low birth weight (z-score<0) as well as high birth weight (z-score≥0), the analyses were repeated after stratification according to birth weight.

Poisson regression was used for weight gain and number of primary care consultations for wheezing illnesses in the groups of infants with at least 12 and 36 months of follow-up and finally in the total group, with the follow-up duration in months used as offset variable.

In order to estimate the persistence of the abovementioned associations, linear regression analysis was used to assess the association between weight gain in the first three months and age and length adjusted FEV_1 and FEF_{25-75}. Neonatal lung function and age at neonatal lung function were considered possible confounders, as well as the abovementioned confounders. All the analyses were repeated with length gain as independent continuous variable.

Results are presented as incidence rate ratios (IRR), indicating relative change in outcome rates, and linear regression coefficients, 95% confidence intervals and

p-values. Associations were considered statistically significant if p-values were <0.05. All analyses were run using SPSS (version 15.0, SPSS Inc., 2007, Chicago USA).

Results

Between December 2001 and January 2009, 1602 infants were included in the study (Figure 1). In the initial phase of the project, no monthly follow-up data on growth were yet collected (97 participants). In 1431 children, data on both weight gain and wheezing symptoms (mean number of returned monthly questionnaires between the 4th and 12th month of life was 7.8 months) were obtained and 1335 children had data on both growth and primary care consultations (median follow-up 38.2 months). Among the 413 children who were five years old, 60 could not be reached. Among the remaining 353 children, 271 (76.8%) children agreed to participate in the follow-up study. Valid follow-up lung function measurements at five years of age were obtained in 235 children (mean age: 5.3 years; SD: 0.2 years). No differences were found between infants with and without data on growth, with and without information on consultations and with and with out lung function measurement at five years of age in

Figure 1. Overview of the study population.

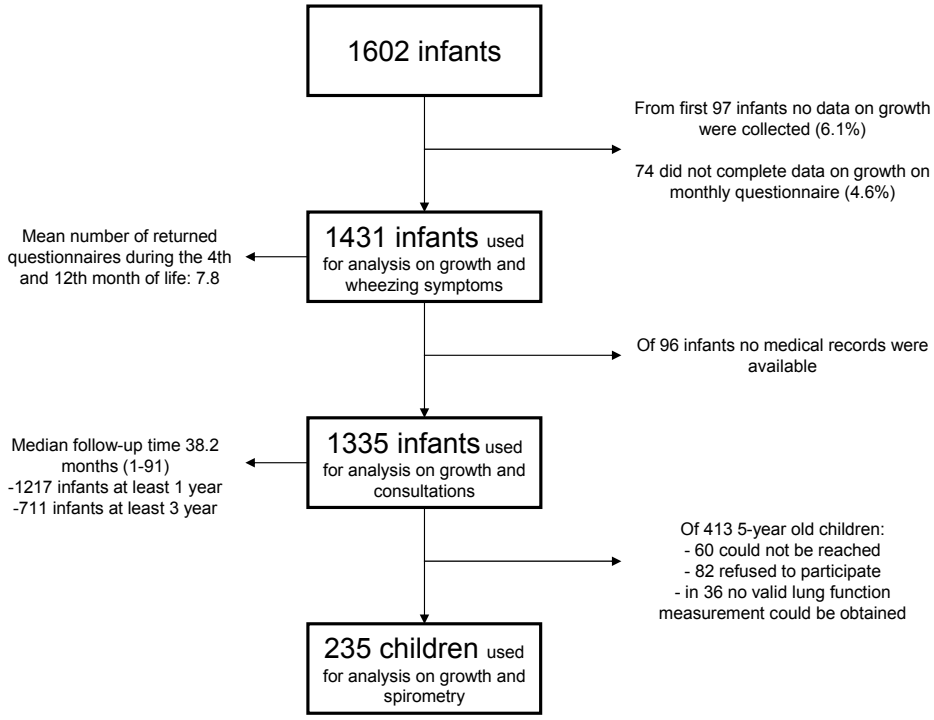


Table 1. Baseline characteristics of total study population by growth pattern.

	Total group N = 1431	**Slow** weight gain N= 338	**Normal** weight gain N=770	**Rapid** weight gain N=323	**Statistics**
Sex (% boys)	48.6	31.7	47.4	69.3	**<0.001[a]**
Birth Weight (mean, in grams)	3525	3859	3500	3237	**<0.001[b]**
Birth Length (mean, in cm)	50.9	51.9	50.9	50.0	**<0.001[b]**
Weight day 90 (mean, in grams)	6069	5703	6042	6516	**<0.001[b]**
Length day 90 (mean, in cm)	61.1	61.1	61.0	61.4	0.119[b]
Gestational age (mean days)	278.6	282.1	278.8	274.5	**<0.001[b]**
Maternal asthma in last 12 months (%)	9.2	7.6	10.0	9.0	0.493[a]
Maternal allergy (at least on of: house dust mite, food, pets or having hay fever) (%)	38.6	35.0	39.2	41.0	0.313[a]
Paternal asthma in last 12 months (%)	6.4	5.8	6.1	7.7	0.632[a]
Paternal allergy (at least on of: house dust mite, food, pets or having hay fever) (%)	37.2	37.4	36.3	39.5	0.696[a]
Exclusive breastfeeding (median, wks)	6.9	8.9	7.1	5.9	0.313[c]
Exclusive breastfeeding first 3 months (%)	42.4	46.2	42.0	39.2	0.194[a]
Breastfeeding (with/without formula milk feeding) first 3 months (%)	62.9	66.3	63.5	58.0	0.085[a]
Siblings (% with at least one)	52.5	59.2	50.3	50.6	**0.019[a]**
Pet ownership during pregnancy (%)	40.4	38.5	40.4	42.4	0.585[a]
Pet ownership after birth (%)	39.6	36.9	39.5	42.6	0.337[a]
Maternal smoking during pregnancy (%)	5.6	4.7	5.6	6.5	0.613[a]
Smoke exposition after birth (%)	12.3	12.1	13.2	10.9	0.798[a]
Maternal higher education (%)	66.5	69.3	65.6	65.8	0.527[a]
Birth season (%)					0.792[a]
Winter	22.8	23.1	23.0	22.0	
Spring	25.4	24.3	24.9	27.6	
Summer	26.8	29.0	25.7	27.2	
Autumn	25.0	23.7	26.4	23.2	
Ethnicity mother (% Western)	90.7	93.3	90.9	87.2	**0.048[a]**
Ethnicity father (% Western)	91.6	94.5	90.6	90.6	0.124[a]

a= Chi-square

b= Anova

c= Kruskal-Wallis test

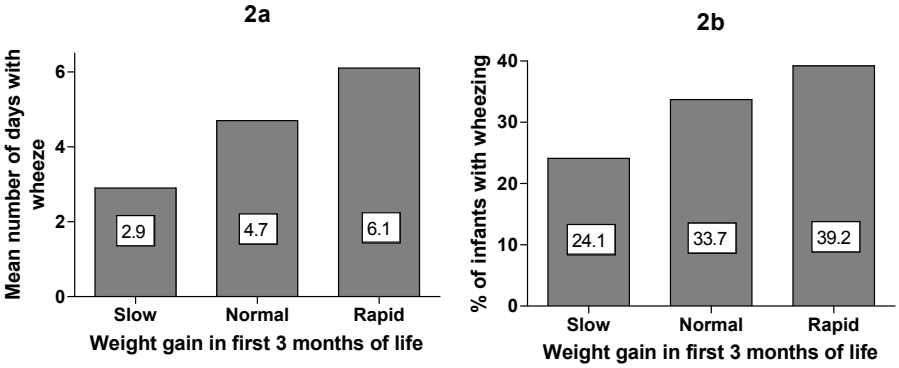
Categories of weight gain: Δ weight z-scores (adjusted for age 90 days) between birth and 3 months. **Slow** weight gain: Δ z-score < -0.67, **Normal** weight gain: Δ z-score ≥ -0.67 and < 0.67, **Rapid** weight gain: Δ z-score ≥ 0.67.

terms of parental allergy, gestational age, gender, siblings, smoking during pregnancy, birth weight, ethnicity of the mother and exclusive breastfeeding in the first quartile (data not shown).
Table 1 shows baseline characteristics for different weight gain patterns. Infants with rapid weight gain were more often male, were born after shorter gestation and had less frequently mothers of western origin and siblings.

Early weight gain pattern and wheezing in the first year of life

Of all infants, 36% had wheezing complaints between the 4th and 12th month of the first year of life and 15% had more than 7 days of wheezing.
With increasing weight gain infants showed more days with wheezing symptoms (figure 2a) and a higher percentage of the children wheezed (figure 2b). Table 2 shows a 36% higher rate of days with wheezing symptoms with every standard deviation higher weight gain, increasing to 44% after adjustment for gender, gestational age and other potential confounders. Similar associations were found within children with high or low birth weight (data not shown).

Figure 2. Mean number of days with wheezing symptoms (2a) and percentage of children with wheezing complaints (2b) in the 4th to12th month of life per weight gain category.



No significant association was found between length gain and days with wheezing symptoms, after adjustment for confounders (IRR 1.03, 95% CI: 0.94-1.12, p 0.576).

Early weight gain pattern and primary care consultations for wheezing illnesses

25.2% of all infants had at least one primary care consultation for wheezing illnesses during the first year of life, and 39.7% of all infants during the first three years of life.

Table 2. Association between weight gain in the first three months of life and days with wheezing symptoms or primary care consultations for wheezing illnesses.

Risk Factor	***Crude***		***Adjusted***	
	IRR (95% CI)	**P-value**	**IRR (95% CI)**	**P-value**
	Days with wheezing symptoms in month 4-12 (number of monthly questionnaires is offset)			
Weight gain† (per 1 z-score increase)	1.36 (1.27-1.45)	**<0.001**	1.35 (1.26-1.45)*	**<0.001**
			1.44 (1.33-1.57)**	**<0.001**
	Primary care visits for lower respiratory illnesses in first year of life			
Weight gain† (per 1 z-score increase)	1.26 (1.17-1.37)	**<0.001**	1.20 (1.10-1.30)*	**<0.001**
			1.24 (1.12-1.38)**	**<0.001**
	Primary care visits for lower respiratory illnesses in first 3 years of life			
Weight gain† (per 1 z-score increase)	1.22 (1.14-1.31)	**<0.001**	1.16 (1.07-1.26)*	**<0.001**
			1.19 (1.08-1.30)**	**0.001**
	Primary care visits for lower respiratory illnesses during total follow-up (follow-up is offset)			
Weight gain† (per 1 z-score increase)	1.19 (1.13-1.26)	**<0.001**	1.12 (1.05-1.18)*	**<0.001**
			1.11 (1.04-1.19)**	**0.002**

IRR: incidence rate ratio. † Differences between z-score for weight at age 3 months (adjusted for the exact age in days) and at birth. * adjusted for sex and gestational age. ** Also adjusted for other potential confounders (maternal smoking during pregnancy, parental smoking after birth, duration of exclusive breastfeeding, parental allergy, siblings, and the ethnicity of the mother).

Table 2 shows that one standard deviation more weight gain was related to a 24% higher rate of primary care consultations for lower respiratory symptoms in the first year of life, a 19% higher rate in the group of children with 3 years of follow-up, and an 11% higher rate in the total group, accounting for follow-up duration. No significant association was found between length gain and primary care consultations for respiratory symptoms (data not shown).

Early weight gain pattern and lung function at the age of five

Mean FEV_1 at the age of five was 1.280 l (SD: 0.177 l) and mean FEF_{25-75} was 1.502 l (SD: 0.386 l). Table 3 shows that, after adjustment for confounders, one standard deviation more weight gain was associated with a significant decrease in FEV_1 (minus 36 ml (-2.8%)) and a significant decrease in FEF_{25-75} (minus 80 ml (-5.3%)).

Table 3. Association between weight gain in the first three months of life and lung function (FEV1 and FEF25-75) at five years of age.

Risk Factor	***Crude***		***Adjusted***	
	Regression coefficient (95% CI)	**P-value**	**Regression coefficient (95% CI)**	**P-value**
	FEV_1 (l) ‡			
Weight gain† (per 1 z-score increase)	−0.025 (−0.044 - −0.005)	**0.014**	−0.035 * (−0.056 - −0.013)	**0.002**
			−0.036 ** (−0.065 - −0.006)	**0.018**
	FEF_{25-75} (l/s) ‡			
Weight gain† (per 1 z-score increase)	−0.059 (−0.111 - − 0.008)	**0.024**	−0.079 * (−0.136 - −0.023)	**0.006**
			−0.080 ** (−0.159 - −0.001)	**0.049**

Beta = linear regression coefficient. † Differences between z-score for weight at 3 months of age (adjusted for the exact age in days) and at birth. ‡ FEV_1 and FEF_{25-75} adjusted for age and length at measurement. * Adjusted for sex and gestational age; **Also adjusted for other potential confounders (maternal smoking during pregnancy, parental smoking after birth, duration of exclusive breastfeeding, parental allergy, siblings, and the ethnicity of the mother).

Discussion

This study shows that rapid weight gain in the first 3 months after birth is associated with more days with wheezing symptoms in the first year of life and decreased lung function at five years of age. Adding to clinical relevance, accelerated weight gain is associated with more primary care consultations for lower respiratory symptoms during the first years of life.

The strength of this study is the large sample size of healthy newborns and the prospective standardized way of data collection. Data on wheezing complaints were collected on a daily basis and we were able to adjust for the most important confounders. However, some methodological considerations should be made. Firstly, information on wheezing symptoms was obtained from questionnaires with parent-reported symptoms, which may be misclassified due to confusion about the distinction between wheeze and snoring or cough[23]. We minimized this by careful parental instruction and the percentage of children with wheezing complaints was similar to other studies[24,25]. Most importantly, the possible misclassification is probably non-differential, and therefore unrelated to weight gain pattern. Secondly, not all parents completed all monthly questionnaires during the first year of life of their child. The mean number of returned questionnaires during the 4th and 12th month was 7.8; however this was slightly lower in the group with rapid weight gain (7.5 questionnaires). In most instances, the last questionnaires were missing, while children more often experienced respiratory symptoms in this period than in earlier months. This might have led to an underestimation of the association between early life rapid weight gain and wheezing. Last, we calculated z-scores instead of using age-related, gender-specific growth charts. We consider the WHISTLER study group representative of the healthy population, as confirmed by the fact that the mean birth weight and weight at three months are in line with the average weights according to (inter)national growth charts[26,27]. Moreover, the relevance of our findings pertains to within group relative growth patterns.

In our cohort wheezing complaints were only prospectively documented during the first year of life. Primary care consultations were obtained for the total follow-up period. The association between rapid weight gain and primary care consultations seemed to be somewhat stronger in the first year than in the following years, suggesting that the effect diminishes with increasing age. One explanation could be that at older age other factors play an increasingly important role in respiratory symptoms and consultations. Follow-up of our cohort will show if the effect of rapid weight gain on respiratory symptoms persists, or disappears during childhood, relative to other causes. Nevertheless, in the 5 year old subgroup that experienced rapid weight gain in the first three months, 32.1% reported wheezing over the last

12 months, which was significantly more often than the children with normal (7.7%) or slow (19.0%) weight gain (X-square: p=0.020). Furthermore, they reported significantly more often to have had a doctor's diagnosis of asthma (18.2% versus 3.3% and 3.5%, X-square: p= 0.001).

Only a few studies investigated the relation between rapid growth gain and wheezing symptoms[13-15]. Although these studies investigated different domains and different periods of weight gain and outcome, they showed similar results. Our results are also in line with studies showing decreased lung function after rapid postnatal growth[16,17].

Our findings could be caused by different mechanisms. According to Barker's hypothesis[28] chronic conditions later in life are due to an unfavourable foetal environment, with retarded growth in utero and catch-up growth after birth. Later studies showed that mainly rapid catch-up growth seems to be a risk factor for future outcomes[29,30]. The 'mismatch hypothesis' proposes that especially the difference between the foetal environment and the environment after birth could result in diseases later in life[31]. One of the other possible explanations is chronic inflammation. Obesity can be seen as a state of chronic low-grade systemic inflammation. Contrary to rapid length gain, especially rapid weight gain was associated with wheezing symptoms. Although not all children with rapid weight gain were obese, there was at least acquisition of adipose tissue. Adipokines, chemokines and other serum factors from adipose tissue could lead to inflammation at other sites[32], such as the airways with consequent wheezing complaints. Since small airways and viral infections play an important role in wheezing in the first year of life[33], our findings may also be explained by disproportional growth, with lung development lagging behind somatic growth.

The results of this study may have implications for clinical practice. Although not all wheezing illnesses will develop into asthma, we believe that reducing overfeeding and rapid weight gain could help diminish the burden of wheezing illnesses in children and their families, and associated burdens to primary health care. However, for definite confirmation randomized comparisons are required.

In conclusion, this study showed that rapid early postnatal weight gain is associated with more wheezing illnesses in the first years of life and reduced lung function at five years of age.

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CHAPTER 4

The effect of implementation of smoke-free legislation on environmental tobacco smoke exposure during pregnancy and neonatal lung function in the Netherlands

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Submitted

Abstract

Rationale: Smoke-free legislation results in improvement of respiratory symptoms and lung function in adults. The Dutch Tobacco Law prohibited smoking in working places and public transport since 2004. In 2008 it was extended to include bars and restaurants.

Objectives: To study the effects of incremental smoke-free legislation in the Netherlands on environmental tobacco smoke exposure (ETS) in pregnant women and on birth weight and lung function of their offspring.

Design: Prospective birth cohort study

Setting: Primary health care centres in a new residential area in the Netherlands.

Participants: Healthy newborns, born between December 2001 and April 2010, covering three consecutive periods with increasing levels of restriction to ETS. Exclusion criteria were gestational age <36 weeks, major congenital abnormalities and neonatal respiratory disease.

Main outcome measures: Percentage of women exposed to ETS during pregnancy, birth weight and newborn lung function (compliance, C_{RS} (ml/kPa), and resistance, R_{RS} (kPa/l/s) of the respiratory system) were compared between the three consecutive subgroups.

Results: 1654 infants were included. The percentage of women reporting ETS exposure declined from 24.8% (124/499) in the 2001-2004 group to 5.5% (24/434) in the 2008-2010 group ($p<0.001$). After adjustment for potential confounders in the respective groups mean birth weight increased (plus 82 gram, $p = 0.017$), median C_{RS} increased (41.8 to 46.3 ml/kPa, $p <0.001$) and median R_{RS} decreased (7.1 to 5.8 kPa/l/s, $p<0.001$).

Conclusion: Incremental implementation of environmental tobacco smoke-banning legislation in the Netherlands was associated with a progressive reduction of tobacco smoke exposure during pregnancy and an incremental increase in birth weight and newborn lung function.

Introduction

Exposure to environmental tobacco smoke (ETS) is associated with adverse health effects, like lung cancer and coronary heart disease in adults [1,2] as well as respiratory symptoms [3,4] and decreased lung function in children.[5] ETS exposure in pregnant women results in reduced birth weight in their offspring,[6] suggesting that passive maternal smoking is a significant source of foetal exposure. The effects of prenatal ETS on newborn lung function have never been studied.

Studies in adults showed beneficial effects of smoke-free legislation on respiratory symptoms,[7-9] lung function[8,9] and myocardial diseases.[10,11] Smoke-free law coverage was shown to be associated with reduced detectable cotinine in youth.[12] A recent Cochrane review recommended the investigation of the impact of smoking bans on health outcomes in young children.[13]

In the Netherlands, the Tobacco Law came into effect in 1990 and prohibited smoking in public premises. In 2004 it was extended to cover workplaces and public transport and in 2008 smoke-free legislation for bars and restaurants was implemented. These measures resulted in a decrease of adults exposed to ETS from 30% (1998-2001) to 18% (2003-2006).[14] Nine months after the smoking-ban in bars and restaurants ETS exposure was reduced from 71% to 17% in bars and from 42% to 4% in restaurants.[15] We studied the effects of incremental smoke-free legislation in the Netherlands on ETS exposure in pregnant women and on birth weight and lung function of their offspring.

Methods

Design and population

This study is part of the prospective birth-cohort study WHISTLER (WHeezing Illness STudy LEidsche Rijn), which has been described elsewhere.[16] It is conducted in a new residential area in Utrecht by the University Medical Centre Utrecht, the Netherlands. Sixty-six percent of all healthy newborns in the area participate in the study. Exclusion criteria were gestational age <36 weeks, major congenital abnormalities and neonatal respiratory disease. At the age of three to eight weeks compliance (C_{RS}) and resistance (R_{RS}) of the respiratory system were measured and questionnaires regarding ETS exposure during pregnancy and confounding factors were filled in. The medical ethics committee of the University Medical Centre Utrecht approved the study. Written parental informed consent was obtained.

Questionnaire

ETS exposure was defined present when the mother confirmed the question "Did you spend time in spaces where other people were smoking during pregnancy?". Active smoking was considered present when the mother confirmed the question "Did you smoke during pregnancy". Birth weight, birth length, gestational age and parity were recorded. Parity was divided into women who gave birth for the first time (nullipara) and women who had previously given birth (multipara). The educational level of the mother was categorized as high (higher vocational or university education) versus moderate-low (primary or primary plus secondary education).

Analyses

Using linear regression, birth weight and length were adjusted for sex, gestational age and parity. C_{RS} and R_{RS} were adjusted for age, length, weight and sex, since these are determinants of lung function. C_{RS} and R_{RS} were non-normally distributed and therefore median values and interquartile ranges are provided. Other continuous variables are presented as mean values with standard deviations. The 2001-2010 cohort was divided into three consecutive birth sub-cohorts according to incremental introduction of the Dutch smoke-ban: basic smoke ban (birth year 2001-2004), smoke ban extended to working places and public transport (birth years 2005-2007) and smoke ban extended to bars and restaurants (birth years 2008-2010) and were mutually compared. Proportional data were compared using chi-square tests. Continuous variables were compared using one-way ANOVA. C_{RS} and R_{RS} were compared using the Kruskal-Wallis-test. Differences between the three groups were determined with the nonparametric Mann-Whitney-U-test with Bonferroni-correction.

Results

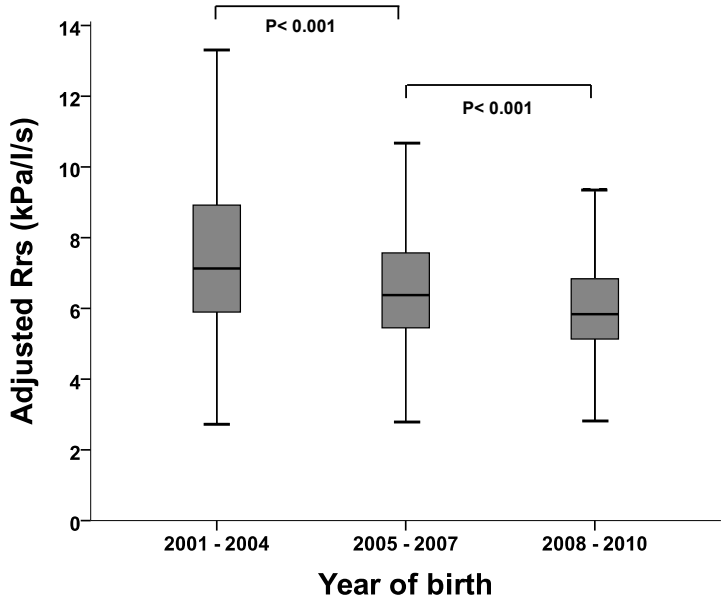
Between December 2001 and April 2010 1654 healthy infants were included in the study. In table 1 the three consecutive subgroups are compared. The number of women exposed to ETS during pregnancy gradually decreased significantly from 24.8% to 5.5%. The decline in ETS exposure in low and high educated women was comparable. After adjustment for possible confounders the infants born between 2008-2010 had an 82 gram higher birth weight ($p < 0.017$) than infants born between 2001-2004. Infants had significantly higher C_{RS} and lower R_{RS} in consecutive year groups. These lung function measurements were performed at significantly higher age, in infants with higher weights and lengths. After adjustment for confounders the increase in C_{RS} and the decrease in R_{RS} stayed significant over the successive year groups (figure 1). Further adjustment of C_{RS} and R_{RS} for active maternal smoking did not change the results.

Table 1. Comparison of demographic characteristics, environmental tobacco smoke (ETS) exposure and newborn lung function in consecutive year groups.

	Year of birth: 2001-2004 (n= 499)	Year of birth: 2005-2007 (n= 721)	Year of birth: 2008-2010 (n= 434)	p-value [d]
Sex (male), n (%)	242 (48.5)	363 (50.3)	191 (44.0)	0.11
Gestational age (days), mean (SD)	279 (10)	279 (9)	278 (9)	0.45
Parity (multipara), n (%)	248 (49.7)	241 (53.4)	227 (52.3)	0.45
Birth weight (g), mean (SD)	3488 (523)	3526 (484)	3549 (511)	0.17
Adjusted birth weight (g) [a], mean (SD)	3490 (457)	3519 (429)	3572 (426) [e]	0.02
Birth length (cm), mean (SD)	51.0 (2.3)	50.8 (2.1)	50.7 (2.3)	0.36
Adjusted birth length (cm) [a], mean (SD)	50.9 (2.0)	50.8 (1.9)	50.9 (2.0)	0.51
Age at visit (weeks), mean (SD)	4.6 (1.3)	4.9 (1.3)	5.5 (1.2)	< 0.001
Weight at visit (g), mean (SD)	4351 (635)	4457 (630)	4633 (641)	< 0.001
Length at visit (cm), mean (SD)	54.4 (2.3)	54.8 (2.5)	55.6 (2.5)	< 0.001
Active smoking in pregnancy, n (%)	42 (8.4)	42 (5.8)	23 (5.3)	0.10
ETS exposure in pregnancy, n (%)	124 (24.8)	65 (9.0)	24 (5.5)	< 0.001
Maternal education (high), n/total (%) [b]	239/383 (62.4)	389/589 (66.0)	243/343 (70.8)	0.06
C_{RS} (ml/kPa), median (IQR)	41.1 (34.2 – 47.8)	46.1 (39.7 – 52.9)	48.6 (41.6 – 55.4)	< 0.001
R_{RS} (kPa/l/s), median (IQR)	7.2 (5.9 – 9.0)	6.4 (5.3 – 7.6)	5.7 (4.9 – 6.7)	< 0.001
Adjusted C_{RS} (ml/kPa) [c], median (IQR)	41.8 (35.6 – 49.7)	46.5 [d] (40.8 – 52.4)	46.3 [e] (40.4 – 52.9)	< 0.001
Adjusted R_{RS} § (kPa/l/s) [c], median (IQR)	7.1 (5.9 – 8.9)	6.4 (5.5 – 7.6)	5.8 (5.1 – 6.8)	< 0.001

a. Adjusted for pregnancy duration, parity and sex;
b. 20.5% of total cases had missing information on "maternal education".
c. Adjusted for age, length and weight at measurement and sex.
d. P-values derived from ANOVA, Kruskal-Wallis-tests and chi-square tests.
e. Only significant difference with year group 2001-2004

Figure 1. Boxplot of resistance of the respiratory system (R_{RS}) adjusted for length, weight, age and sex.



Discussion

This study showed that incremental implementation of environmental tobacco smoke-banning legislation in the Netherlands was associated with a progressive reduction of ETS exposure during pregnancy and a dose-dependent increase in birth weight and newborn lung function. This is the first study that focused on reduction of ETS exposure in pregnant women and improvement of infant health related to smoke-free legislation and health campaigns. The observed reduction in ETS exposure is in line with a prior report showing a decline of ETS exposure in the Dutch population in the same period.[14] Former studies found associations between smoking-bans in bars and increased lung function in bartenders.[8,9] Besides the beneficial effect of smoke-free legislation on lung function of directly exposed persons, this study adds that diminished foetal ETS exposure has a beneficial effect on lung function in newborns as well.

Some methodological factors need to be considered. Firstly, the number of women exposed to ETS could be an underestimate, because we used recall data and not direct cotinine measurements. Underestimation by underreporting of ETS exposure could also be induced by the health campaigns that notify ETS exposure as inappropriate behaviour. This may have resulted in an overestimation of the decline in reported ETS exposure. Although these limitations prohibit calculation of direct dose-response

relationships in our study, the incremental smoking-ban in the consecutive periods was clearly associated with gradual improvements of neonatal lung function.
Secondly, there was a borderline significant increase in the percentage of women with high education, who are less exposed to ETS, over the consecutive periods. This increase could partially explain the observed decline in ETS exposure. However, as the percentage of women exposed to ETS showed a comparable decline in both low and high educated women, the increasing number of women with high education cannot be the explanation for the decrease in ETS exposure. Furthermore, the rising proportion of high educated women cannot explain the improvement in lung function over time, as there is no evidence that maternal education affects newborn lung function.
Our present results have important implications for the respiratory health of infants and children. Decreased newborn lung function is associated with a higher risk of wheezing illnesses in the first years of life.[17-21] In our cohort, we recently showed that each standard deviation increase in R_{RS} was associated with an almost doubled risk of wheezing during a respiratory infection with human rhinovirus.[22] This suggests that the found decrease in R_{RS} (half a standard deviation) due to smoke-free regulations could result in a marked reduction in wheezing during respiratory virus infections.
The "prevention paradox" describes that population-based preventive measures may bring only little benefit to individuals.[23] However, the incidence of wheezing in the first year of life ranges from 25-60 percent [24] and is associated with significant health care consumption and drug prescriptions.[25] Many children will profit from the beneficial effects of reduced ETS exposure during pregnancy, and smoke free-legislation will considerably influence the incidence of wheeze in childhood. Prenatal smoke exposure induces other health risks, e.g. a higher systolic blood pressure at birth,[26] obesity in childhood [27] and a higher carotid artery intima-media thickness in young adults.[28] It might be expected that the decline in women exposed to ETS during pregnancy will therefore result in more health benefits in the next generation. A recent study showed that recognition of the beneficial effects of a smoke-free environment on newborns' health results in more smoke-free homes.[29] We therefore strongly advocate ratification of the WHO Framework Convention on Tobacco Control to implement tobacco control measures in all countries worldwide.

Acknowledgements

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CHAPTER 5

Temporal effects of tobacco smoke exposure on lung development in early life

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Abstract

Objective It is important to know whether dcreased lung function caused by prenatal smoke exposure is permanent or wanes in childhood. We investigated the effect of prenatal and postnatal smoke exposure on lung function change between birth and five years.

Methods In the prospective birth cohort study WHISTLER, compliance and resistance of the respiratory system (C_{RS} and R_{RS}) were measured at neonatal age and spirometry at five years. Change in lung function was calculated as FEV_1 z-score minus C_{RS} z-score (Δ-Z-FEV_1-C_{RS}) and the negative z-score of R_{RS} (Δ-Z-FEV_1-R_{RS}). Questionnaires on smoke exposure were obtained.

Results Measurements were performed in 225 children (5.4 yrs). Prenatal smoke exposure resulted in decreased neonatal lung function (passive smoking: linear regression coefficient Z-C_{RS}: -0.27 SD, p= 0.100; active smoking: Z-R_{RS}: +0.75 SD, p =0.022) and a subsequent increase in lung function (Δ-Z-FEV_1-C_{RS}: 0.48 SD/5 yr, p = 0.045; Δ-Z-FEV_1-R_{RS}, 0.36 SD/5 yr, p= 0.186). Postnatal smoke exposure resulted in a lung function decline (Δ-Z-FEV_1-C_{RS}: -0.63 SD/5 yr, p= 0.038; Δ-Z-FEV_1-$R_{RS:}$ -0.40 SD/5 yr, p= 0.244), although resistance estimates were not statistically significant. Children both prenatally and postnatally exposed to smoke showed less increase in lung function compared to children prenatally exposed only.

Conclusion The negative effects of prenatal smoke exposure on lung function seem to diminish in early childhood, although this waning effect is less in children postnatally exposed to tobacco smoke. Postnatal smoke exposure reduces lung function. Both prenatal and postnatal smoke exposure are detrimental to newborns lung function development.

Introduction

The phenomenon of longitudinal stability of risk factors over time is called 'tracking'[1]. There seems to be substantial 'tracking' of lung function from birth into infancy [2;3] and childhood [4-6] and from childhood into adulthood[1;7-9]. Infants with decreased lung function at birth might have significant health risks in later life because decreased lung function is a major clinical indicator of overall mortality in adults [10].
Antenatal influences like prenatal tobacco smoke exposure are associated with impaired lung function of children at birth [11] and might therefore have long-term influences on childhood and adult health. A meta-analysis, combining data from six cross-sectional studies, found decreased lung function in children aged 6-12 exposed to maternal smoking in utero [12]. On the other hand, the development of the lungs continues during the first years of life with increasing numbers of alveoli and growth of the conducting airways. Therefore, one might hypothesize that the lungs could develop some "catch-up" growth, when in utero exposure to smoking immediately ceases at birth and lung growth proceeds. Longitudinal studies into the relationship between prenatal smoke exposure and development of lung function in later life are scarce. To our knowledge, the only longitudinal study available is the East Boston study, which showed a smaller reduction in lung function at the age of 18 months [13] than shortly after birth [14] in children prenatally exposed to maternal smoking. This suggests that loss of neonatal lung function due to prenatal smoke exposure is not necessarily permanent and that the effect can wane during the first years of life.
We aimed to investigate the effect of prenatal smoke exposure on neonatal lung function and whether the effect persists or diminishes during childhood. In a prospective birth cohort study we analyzed the association between prenatal smoke exposure, neonatal lung function and the change in lung function between birth and five years of age. Secondly, we assessed the influence of postnatal smoke exposure on change of lung function in early life.

Methods

Study design and study population

Children were participants of the WHeezing Illnesses STudy LEidsche Rijn (WHISTLER), a prospective birth cohort study on determinants of wheezing illnesses, which started in December 2001 [15]. Exclusion criteria were gestational age <36 weeks, major congenital abnormalities and neonatal respiratory disease. Briefly, lung function, length and weight were measured between 3 and 8 weeks of age, before any respiratory infection occurred. At the age of five, spirometry, height and weight were

measured. The study was approved by the medical ethics committee of the University Medical Center Utrecht and parents gave written informed consent.

Pulmonary function measurements

Single occlusion technique

Resistance (R_{RS}) and compliance (C_{RS}) of the total respiratory system were measured, using the single occlusion technique [16] during natural sleep. Decreased C_{RS} and increased R_{RS} indicate decreased lung function. Further details about the lung function measurement were previously described [17].

Flow/volume measurements

Forced vital capacity (FVC) manoeuvres were obtained using a heated Lilly head pneumotachometer system (Viasys Healthcare, Hochberg, Germany). Measurements were BTPS corrected and performed conform the latest ATS/ERS statement for lung function measurements in preschoolers [18]. At least two reproducible flow-volume curves were obtained. The largest forced expiratory volume in 1 second (FEV_1) was selected and maximal mid-expiratory flow (FEF_{25-75}) was obtained from the curve with highest sum of FEV_1 and FVC.

Definitions

Prenatal smoke exposure was assessed with a questionnaire at the first visit. "Active maternal smoking during pregnancy" was defined as self-reported smoking during both semesters of pregnancy. "Passive maternal smoking during pregnancy" was considered present when the mother reported regular smoke exposure during the entire pregnancy without active smoking during pregnancy. "Prenatal smoke exposure" was defined as active and/or passive maternal smoking during pregnancy. "Postnatal smoke exposure" was assessed with a questionnaire at the five year visit and considered present when mother and/or father confirmed the question "do you smoke?".

Analysis

Since lung function was measured with different techniques at different ages, z-scores of lung function were computed to transform the measurements to lung function ranks within the distributions. Measurements were not performed at exactly the same age in all children. Therefore all regression analyses were first adjusted for age. Further analyses were performed with additional adjustment for height, weight and sex.

First, the association between prenatal smoke exposure and neonatal lung function was assessed, using univariate linear regression analysis with C_{RS} and R_{RS} z-scores (Z-C_{RS}, Z-R_{RS}) as dependent variables.
Second, the effect of prenatal and postnatal smoke exposure on change in lung function was investigated. Children were categorised as "prenatal smoke exposure only", "postnatal smoke exposure only" and " both prenatal and postnatal smoke exposure" and compared to a reference group "no smoke exposure", using univariate linear regression analysis with dummy variables for these exposure categories. The dependent variable in these analyses was change in lung function over the first five years. Change in lung function was computed as difference in FEV_1 z-score (Z-FEV_1) and Z-C_{RS} (Delta-Z-FEV_1-C_{RS}) and difference between Z-FEV_1 and -since low R_{RS} corresponds with high lung function- the negative value of Z-R_{RS} (Delta-Z- FEV_1-R_{RS}). In the same way, differences between z-scores of FEF_{25-75} (Z-FEF_{25-75}) and Z-C_{RS} and the negative value of Z-R_{RS} were computed (Delta-Z-FEF_{25-75}-C_{RS} and Delta-Z-FEF_{25-75}-R_{RS}). Using the negative value of Z-R_{RS} implies that positive changes in lung function can be interpreted as increase in lung function and negative changes as decrease in lung function. Change in lung function of children categorised as "prenatal smoke exposure only" or "postnatal smoke exposure only" were compared with children categorised as "no smoke exposure", by plotting mean lung function z-scores at 0 (Z-C_{RS}, negative Z-R_{RS}) and 5 years of age (Z-FEV_1 and Z-FEF_{25-75})". Unless mentioned otherwise, z-scores are graphically shown adjusted for age, as well as adjusted for age, height, weight and sex.

Results

Study population

Among the 454 eligible children who were five years old and had successful neonatal lung function measurements, 67 could not be reached. Among the remaining 387 children, 261 (67.4%) children agreed to participate in the follow-up study. Valid follow-up lung function measurements at five years of age were obtained in 225 children. Failure to obtain valid lung function measurements was mainly due to irreproducible curves (n=10), technically inacceptable measurements (n=20) or unwillingness to participate (n=6). Data on postnatal smoke exposure were missing in 32 children. Table 1 summarizes the characteristics and lung function data of the study population at both visits.
Prenatal smoke exposure was present in 59 children: 10 (4.4%) children had a mother who smoked during pregnancy and the mothers of 49 children were exposed to smoke

Table 1. Characteristics of study population at 0 and 5 years of age. Mean values are presented with standard deviations between parentheses.

	At birth (n=225)	At 5 years (n=225)
Length/ height (cm)	54.4 (2.2)	114.6 (5.0)
Weight (kg)	4.3 (0.6)	20.1 (2.8)
Age (weeks/years)	4.5 (1.2)	5.4 (0.3)
Sex (% female)	55.6	55.6
C_{RS} (ml/kPa)	41.6 (11.4)	
R_{RS} (kPa/l/s)	7.6 (2.4)	
FEV_1 (l)		1.3 (0.2)
FEF_{25-75} (l/s)		1.5 (0.4)
Active maternal smoking in pregnancy (%)	4.4	
Passive maternal smoking in pregnancy (%)	24.9	
Postnatal smoke exposure (%)		19.7

from others only (passive maternal smoking). Postnatal smoke exposure was present in 19.7% of the children.

Relation between prenatal smoke exposure and level of neonatal lung function

The associations between prenatal smoke exposure and Z-C_{RS} and Z-R_{RS} are demonstrated in Table 2. Univariate regression analysis showed a significant positive relation between prenatal smoke exposure and Z-R_{RS} and a borderline significant association with lower values of Z-C_{RS}. After adjusting for length, weight and sex the association between active maternal smoking during pregnancy and Z-R_{RS} became stronger.

Relation between *prenatal* smoke exposure and 5-year postnatal change in lung function

Children with "prenatal smoke exposure only" showed an increase in Delta-Z-FEV_1-C_{RS} (0.48 SD/5 yrs, p= 0.045) and Delta-Z-FEV_1-R_{RS} (0.36 SD/5 yrs, p= 0.186), pointing to catch-up growth of lung-function in children in which smoke exposure was limited to the prenatal period only. No significant increase in Delta-Z-FEF_{25-75}-C_{RS} and Delta-Z-FEF_{25-75}-R_{RS} was found. Children with "both prenatal and postnatal smoke exposure" showed less increase in most lung function measures than children exclusively prenatally exposed to tobacco smoke.

Mean change of lung function z-scores between 0 and 5 years of age of children categorised as "prenatal smoke exposure only" are plotted and compared to children categorised as "no smoke exposure" in figure 1. The increase in lung function z-scores after prenatal smoke exposure persisted after adjustment for height, weight and sex.

Table 2. Relation between prenatal smoke exposure and z-scores of compliance (Z-C_{RS}) and resistance (Z-R_{RS}) of the respiratory system: univariate linear regression, adjusted for age and adjusted for age, length, weight and sex (fully adjusted). Reference group is "no prenatal smoke exposure". Decreased Z-C_{RS} and increased Z-R_{RS} indicate decreased lung function.

	Z-C_{RS}					
	Adjusted for age			**Fully adjusted**		
Prenatal smoke exposure:	**Beta**	**95% CI**	**p**	**Beta**	**95% CI**	**p**
Active and/or passive smoking of mother	-0.22	-0.52 – 0.08	0.151	-0.24	-0.55 – 0.07	0.134
o **Active smoking**	0.03	-0.61 – 0.66	0.935	-0.08	-0.73 – 0.57	0.809
o **Passive smoking only**	-0.27	-0.59 – 0.05	0.100 #	-0.27	-0.60 – 0.06	0.112
	Z-R_{RS}					
	Adjusted for age			**Fully adjusted**		
Prenatal smoke exposure:	**Beta**	**95% CI**	**p**	**Beta**	**95% CI**	**p**
Active and/or passive smoking of mother	0.10	-0.20 – 0.40	0.522	0.19	-0.12 – 0.50	0.232
o **Active smoking**	0.75	0.11 – 1.39	0.022 *	0.95	0.31 – 1.59	0.004 *
o **Passive smoking only**	0	-0.36 – 0.28	0.819	0.03	-0.30 – 0.36	0.868

$p \leq 0.10$; * $p \leq 0.05$

Children categorized as "prenatal smoke exposure only" finished at higher ranks in comparison with the reference category "no smoke exposure", although the difference in ranks was smaller when z-scores were adjusted for height, weight and sex.

Relation between *postnatal* smoke exposure and 5-year postnatal change in lung function

Postnatal tobacco smoke exposure was associated with a decrease in lung function over the first 5 years of life, with more pronounced effects after adjustment for height, weight and sex (Delta-Z-FEV_1-C_{RS}: -0.63 SD/5 yrs, p= 0.038; Delta-Z-FEV_1-$R_{RS:}$ -0.40 SD/5 yrs, p= 0.244; Delta-Z- FEF_{25-75}-C_{RS}: -0.95 SD/5 yrs, p=0.007; Delta-Z- FEF_{25-75}-R_{RS}: -0.73 SD/yrs, p= 0.035).

Figure 2 shows the mean change of lung function z-scores between 0 and 5 years of age of children categorised as "postnatal smoke exposure only", comparing them with children categorised as "no smoke exposure". The decrease in lung function z-scores after postnatal smoke exposure was larger after adjustment for height, weight and sex and children categorized as "postnatal smoke exposure only" finished at lower ranks in comparison with the reference category "no smoke exposure".

Effect of smoke exposure on FEF_{25-75} compared to FEV_1

When adjusted Z-FEF_{25-75} instead of adjusted Z-FEV_1 was used as outcome measure of lung function at 5 years, children exposed to tobacco smoke in utero showed less

Figure 1. "Prenatal smoke exposure and 5-year postnatal change in lung function".
Change in lung function z-score in children with "prenatal smoke exposure only", between 0 (C_{RS}; 1A and negative R_{RS}; 1B) and 5 years of age (FEV_1). Grey lines indicate z-scores adjusted for age; black lines indicate z-scores adjusted for age, height, weight and sex. Dashed lines represent reference "no smoke exposure".

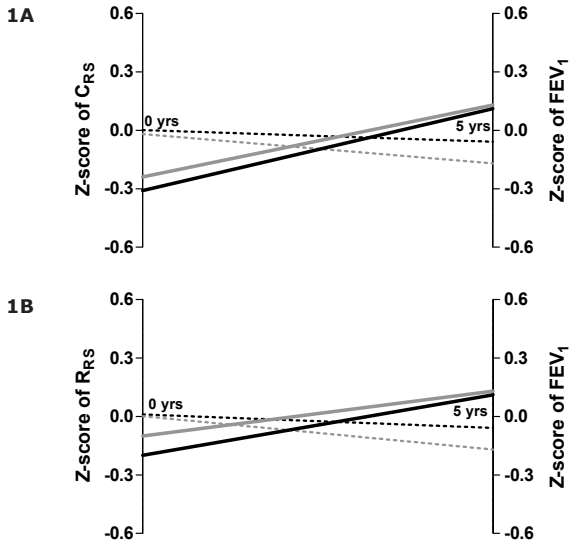


Figure 2. "Postnatal smoke exposure and 5-year postnatal change in lung function".
Change in lung function z-score in children with "postnatal smoke exposure only", between 0 (C_{RS}; 2A and negative R_{RS}; 2B) and 5 years of age (FEV_1). Grey lines indicate z-scores adjusted for age; black lines indicate z-scores adjusted for age, height, weight and sex. Dashed lines represent reference "no smoke exposure".

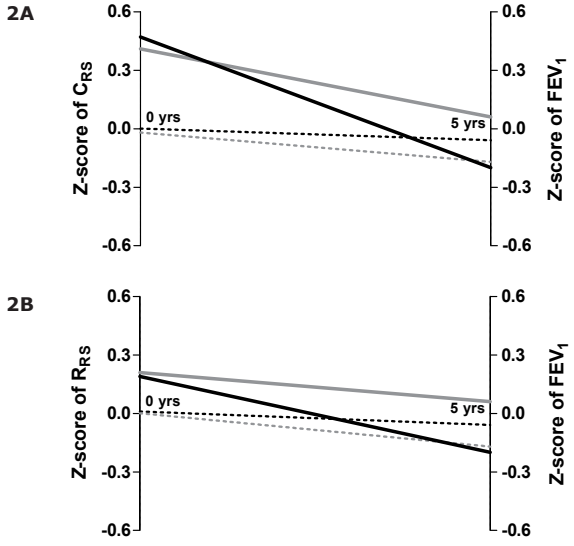
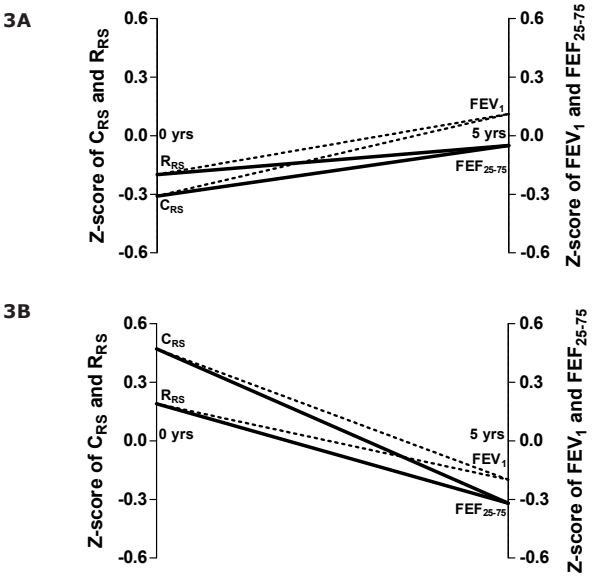


Figure 3. "Difference between effect of smoke exposure on FEF_{25-75} and FEV_1"
Change in adjusted lung function z-scores between 0 (C_{RS} and negative R_{RS}) and 5 years of age (FEV_1 and FEF_{25-75}), in children with "prenatal smoke exposure only" (3A) and "postnatal smoke exposure only" (3B). Dashed lines show change in FEV_1; solid lines show less increase (3A)/ more decrease (3B) in FEF_{25-75}.



increase in lung function (figure 3A). Furthermore, postnatal smoke exposure resulted in a more pronounced reduction in FEF_{25-75} than in FEV_1 (figure 3B).

Discussion

This study showed that prenatal smoke exposure had a negative effect on neonatal lung function but this effect waned throughout the first five years of life. However, postnatal smoke exposure led to a decrease in lung function and children exposed both prenatally and postnatally to tobacco smoke showed less increase in lung function than children with only prenatal smoke exposure. This might imply that ongoing smoke exposure after birth will result in less "catch-up" growth of lung function compared to children without postnatal tobacco smoke exposure.
To our knowledge, this is the first longitudinal study to report the influence of in utero smoke exposure on the change of lung function in the first five years of life. Infants who were prenatally exposed to tobacco smoke showed decreased neonatal lung function. These results are consistent with other studies in full term healthy neonates[14;19-21], full term high-risk newborns [22] and preterm infants [23] and are also in line with findings in animals [24].

The negative effect of prenatal smoke exposure on lung function did not seem to be permanent, since children exposed to tobacco smoke in utero showed an increase in lung function during the first five years of life. This is in contrast with studies that suggested that the harmful effect of prenatal tobacco smoke exposure on lung function persists into childhood [12;25;26]. In these studies small deficits in lung function were found in children exposed to tobacco smoke in utero. However, these were all cross-sectional studies, without measurements of lung function soon after birth and therefore these studies were not capable to demonstrate that the found loss of lung function was of the same magnitude at birth and constant over the years. To determine if the effect of prenatal smoke exposure is persistent or reversible, follow-up studies estimating the relationship between prenatal smoke exposure and change in lung function are necessary. Our results are in line with the East Boston study that found that reductions in lung function caused by prenatal smoke exposure diminished in the first 18 months of life from 17% to 5% [13;14], which implies that the effect dilutes throughout infancy.

When FEF_{25-75} instead of FEV_1 was used as outcome measure, postnatal smoke exposure had a larger negative effect on Delta-Z-FEF_{25-75}-C_{RS} and Delta-Z-FEF_{25-75}-R_{RS} than on Delta-Z-FEV_1-C_{RS} and Delta-Z-FEV_1-R_{RS}. Furthermore, there was less increase in FEF_{25-75} in children exposed to tobacco smoke in utero. The fact that smoke exposure affects FEF_{25-75} more then FEV_1 is consistent with existing literature; Moshammer et al. found that smoking during pregnancy was associated with larger decreases in FEF_{25-75} (4%) than FEV_1 (1%) [12].

We speculate that the improvement of lung function during the first years of life in infants exposed to tobacco smoke in utero could be explained by "catch-up" growth of lung function. There is increasing support of "catch-up" lung growth in children when their lung disease is no longer active and in experimental settings it was shown that the rate of lung growth can be accelerated in the postnatal period [27]. Furthermore, after cessation of smoking lung function improved in adults [28;29], suggesting that a certain degree of recovery is possible.

The association between prenatal smoke exposure and rise in lung function throughout the first five years of life persisted when height/length, weight and sex were added to the model. Therefore, the improvement of neonatal lung function cannot be explained by "catch-up" body growth in neonates whose mothers smoked during pregnancy.

We have shown that there was "catch-up" growth of lung function after prenatal smoke exposure. On the other hand, we also showed that postnatal smoke exposure resulted in decreased lung function. Furthermore, children with "both prenatal and postnatal smoke exposure" had less increase in lung function than children who were only prenatally exposed to tobacco smoke. Therefore we speculate that there could

be more "catch-up" growth of lung function when there would be no ongoing smoke exposure after birth in children prenatally exposed to tobacco smoke.
At the age of five, the lung function of children exposed to tobacco smoke in utero exceeded the mean lung function of children who were never exposed to tobacco smoke. This excessive "catch-up" growth of lung function in children prenatally exposed to tobacco smoke could have negative results on respiratory complaints.
In comparison with the negative effect of excessive "catch-up" body growth on cardiovascular diseases [30], we speculate that extreme growth of the lungs could result in more respiratory problems in later life. A longer follow-up period is needed to investigate the possible clinical implications.
The major strength of this study is the longitudinal assessment of change in lung function after prenatal smoke exposure. However, the present study may have been influenced by methodological factors. First, information on prenatal smoke exposure was obtained using questionnaires shortly after birth and was not confirmed by cotinine measurements in the pregnant women. This may have resulted in an underestimation of the number of children exposed to tobacco smoke in pregnancy and possibly in an underestimation of the associations.
Additionally, data on postnatal smoke exposure were missing in 14% of the children. However, there were no significant differences in lung function at five years of age or respiratory complaints between children with and without data on postnatal smoke exposure. Therefore we expect the missingness to be random and not to have influenced the results.
Furthermore, we measured different indices of lung function at different ages. FEV_1 and FEF_{25-75} are able to detect decrease in lung function caused by smoke exposure. A possible disadvantage of the single occlusion technique is that it measures the compliance and resistance of the total respiratory system, including upper airways and chest wall[16], and therefore it might be less sensitive to detect differences in lower airway function. Nevertheless, we found a significant increase in R_{RS} and a significant decrease in C_{RS} in children prenatally exposed to tobacco smoke. Consequently, we infer that both techniques are sensitive and accurate enough to detect loss of lung function caused by smoke exposure.
Summarizing, we have clearly shown the detrimental effects of prenatal tobacco smoke exposure on neonatal lung function. Postnatal alleviation of that exposure seems to result in pulmonic "catch-up" growth and perhaps even overgrowth in early childhood. Postnatal smoking exposure also clearly results in adverse lung function development in early childhood. While smoke exposure of fetus and young children is harmful with respect to many health aspects, our findings add that it severely affects both the fetal and infant respiratory system. Cessation of smoking in these sensitive periods of life is always much to the benefit of the child's respiratory system.

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CHAPTER 6A

Reference values for paediatric pulmonary function testing: the Utrecht dataset

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Abstract

Background

Since populations evolve, measurement protocols and equipment improve and analysis techniques progress, there is an ongoing need to reassess reference data for pulmonary function tests. Furthermore, reference values for total lung capacity and carbon monoxide diffusion capacity are scarcely available in children. We aimed to provide updated reference equations for most commonly used pulmonary function indices in Caucasian children.

Methods

In the 'Utrecht Pulmonary Function Reference Data Study' we collected data in Caucasian children aged 2-18 years. We analyzed them using the 'Generalized Additive Models for Location Scale and Shape' (GAMLSS) statistical method.

Results

Measurements of interrupter resistance (R_{int}) (n=877), spirometry (n=1042), bodyplethysmography (n=723) and carbon monoxide diffusion/helium dilution (n=543) were obtained in healthy children. Height (or the natural logarithm of height) and age (or the natural logarithm of age) were both significantly related to most outcome measures. Also sex was a significant determinant, except for RV, RV/TLC, FRC_{pleth}, $Raw_{0,5}$, R_{int} and FEF values. The application of previously published reference equations on the study population resulted in misinterpretation of pulmonary function.

Conclusion

These new paediatric reference equations provide accurate estimates of the range of normality for most commonly used pulmonary function indices, resulting in less underdiagnosis and overdiagnosis of pulmonary diseases.

Introduction

Pulmonary function measurements are important in diagnosis and follow-up of respiratory diseases and their interpretation is highly dependent on reference equations. Regular updating of reference values is advocated [1]. Furthermore, reference values for total lung capacity and carbon monoxide (CO) diffusion capacity are scarcely available in children, while reference values for interrupter resistance (R_{int}) do not cover the entire paediatric age range [2].

The previously published reference equations might be inappropriate for today's paediatric population for several reasons, thus resulting in misinterpretation of pulmonary function. Firstly, reference data, which are often collected decades ago, could be outdated due to evolvement of the paediatric population because of better nutrition and health. Temporal changes in growth and maturation (secular trend) [3] may have influenced pulmonary function. Furthermore, measurement protocols improved and analytical techniques progressed. Secondly, the inclusion of age as a determinant of pulmonary function was often disregarded, although including age results in better fitting reference equations [4,5]. Thirdly, reference values for lung volumes and diffusion capacity were often derived from relatively small samples [6] resulting in less accurate equations. Furthermore, the latest study on lung volumes in Caucasian school children was published in 1993 [7] and the most recently published reference values for lung volumes were obtained in non-Caucasian populations [8,9]. The usefulness of these values in Caucasian children is limited because of ethnic differences in pulmonary function [10]. Finally, R_{int} is considered an alternative measure in children unable to perform a forced expiration (e.g. with developmental or neuromuscular disorders), but even the most recently published reference values for this technique do not cover the whole paediatric age range [2]. Reference values for children aged 13-18 are needed since extrapolation beyond the intended age range is discouraged [1].

Some of the abovementioned disadvantages can be overcome by collating and reanalyzing old data sets to provide new reference equations. In this way, sample size can be augmented, new statistical methods can be applied and age can be included, as shown for spirometry by Stanojevic et al.[5,11]. Nevertheless, the disadvantages such as improvement in health status and changes in measurement protocols and equipment persist. Ideally, new reference values for pulmonary function tests would be prospectively obtained, using the latest standardized protocols.

To prevent misinterpretation and avoid misdiagnosis of pulmonary disease in clinical practice, we aimed to generate new reference values for spirometry, lung volumes, airway resistance, CO diffusion capacity and R_{int} in children of European descent aged 2-18 years. Therefore we prospectively collected pulmonary function measurements,

using standardized measurement protocols and the 'Generalized Additive Models for Location Scale and Shape'(GAMLSS) statistical method [12].

Methods

The cross-sectional "Utrecht Pulmonary Function Reference Data Study" was performed by the Department of Paediatric Pulmonology, University Medical Center Utrecht, Netherlands and approved by the Medical Ethics Committee. Data were collected by experienced paediatric pulmonary function technicians between January 2004 and April 2009 at ten randomly selected primary and secondary schools. R_{int} was also measured at 4 kindergartens.
Children aged 2-18 were eligible. Informed consent was obtained from subjects and/or parents. Exclusion criteria were: doctor's diagnosis of asthma (ever), current use of asthma medication, wheezing in previous 12 months, cystic fibrosis, past thoracic surgery, neuromuscular disease, current active smoking, birth weight < 2000 gram or two non-European parents. Measurements were postponed for at least 6 weeks in children with signs of a respiratory tract infection.

Measurements

Measurements were performed in a mobile pulmonary function lab. Test order was: R_{int}, body plethysmography, spirometry and carbon monoxide (CO) diffusion/helium (He) dilution. Only measurements complying to ATS/ERS guidelines (and updated versions) [13-18] were analyzed. The complete methodology is described in the online depository.

Anthropometric measurements

Height and weight were recorded to the nearest 0.1 centimetre and kilogram using a bodymeter measuring tape with wall stop and electronic weighing scale (Seca, Hamburg, Germany).

Interrupter resistance

Expiratory R_{int} measurements were obtained in children aged 2-18, using MicroRint (Micro Medical Limited, Kent, UK). Median R_{int} was calculated from at least 5 acceptable interruptions.

Body plethysmography

Body plethysmography was performed in children aged 6-18 using a variable pressure plethysmograph (MasterScreen Body, Cardinal Health, Hoechberg, Germany). If 3

technician-accepted functional residual capacity (FRC_{pleth}) measurements agreeing within 5% were unfeasible, 2 measurements agreeing within 5% were considered sufficient. Mean tidal volume (VT), airway resistance ($Raw_{0.5}$ and Raw_{tot}), FRC_{pleth} and expiratory residual volume (ERV_{pleth}) and the highest inspiratory vital capacity (IVC_{pleth}) were recorded. Residual volume (RV_{pleth}) and total lung capacity (TLC_{pleth}) were calculated.

Spirometry

Spirometry was performed in children aged 4-18 using a heated Lilly head pneumotachometer (Cardinal Health, Kleve, Germany). Three technician-accepted flow-volume curves were obtained with FEV_1 and FVC agreeing within 5%. The largest forced expiratory volume in 0.5 or 1 second ($FEV_{0.5}$ or FEV_1), forced vital capacity (FVC) and peak expiratory flow (PEF) were selected. Maximal expiratory flows when 25%, 50%, 75% of FVC is expired (FEF_{25}, FEF_{50}, FEF_{75}) and maximal mid-expiratory flow (FEF_{25-75}) were obtained from the curve with highest sum of FEV_1 and FVC.

Single breath CO diffusion and He dilution

Diffusion capacity was assessed with single breath CO diffusion ($D_{L,CO}$) and lung volumes (alveolar volume (VA), RV_{He}, TLC_{He}, FRC_{He}, IVC_{He}) with single breath He dilution (MasterScreen Diffusion, Cardinal Health, Hoechberg, Germany) in children with FVC of 1.5 L or above. Mean $D_{L,CO}$ was calculated from 2 measurements with maximally 10% difference. For $D_{L,CO}$ values we made the assumption of normal haemoglobin concentration in the study population.

Data analysis

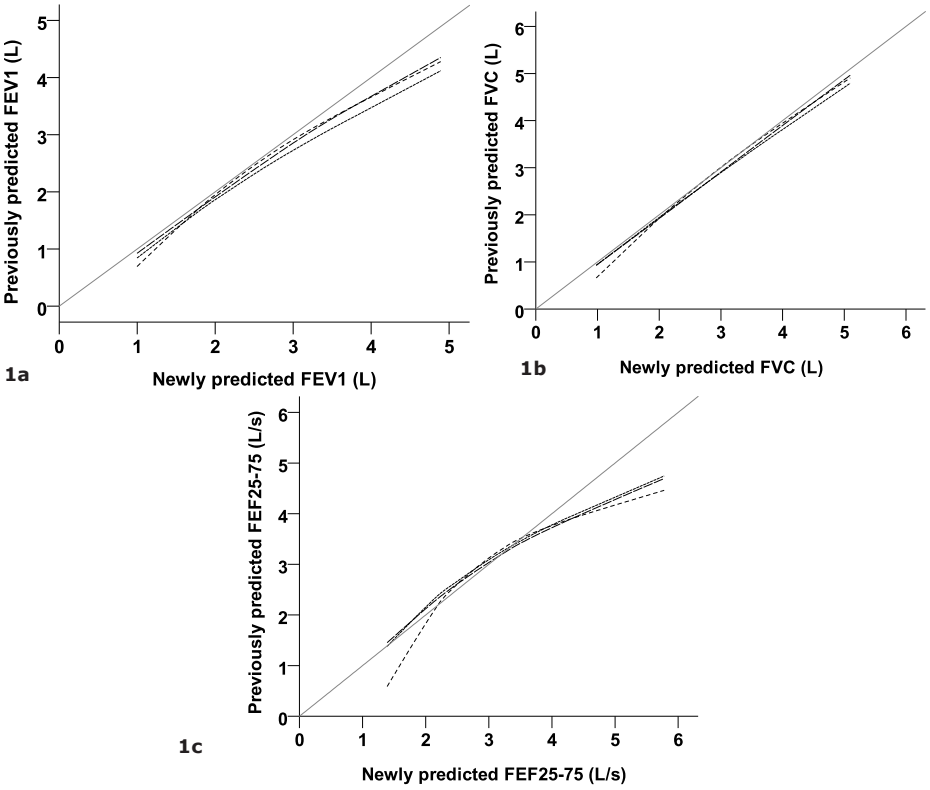
Data were analyzed using GAMLSS (see online supplement for details) [12]. GAMLSS models location (median or mean, *mu*), variability (*sigma*) and dependent on the distribution chosen, skewness (*nu*) and kurtosis (*tau*). Age, height and sex and their two-way interactions were considered explanatory variables. Eligible distributions were: normal, Box-Cox-Cole-Green (BCCG) and Gamma. Reference equations were compared with those mostly used in Europe and North-America [5,19-21].

Results

Reference population

The eligible population comprised all children aged 2-18 years (around 4500) attending the schools and kindergartens, which participated in this study. Informed consent was obtained in 1582 children. For the following reasons not all pulmonary

Figure 1. FEV_1 (1a), FVC (1b) and FEF_{25-75} (1c). Reference values for girls (n=515) predicted by Zapletal et al. [21](·····), Hankinson et al. [19] (- - - -) and Stanojevic et al. [5](— — —) are plotted against newly predicted reference values. The grey line represents the line of identity (perfect agreement).



function tests could be assessed in every child: age related exclusion (see methods section), measurements did not meet ATS/ERS criteria, technical problems with equipment (e.g. strong wind, making bodyplethysmography measurements impossible), shortage of time or unwillingness to participate. Figure 1 in the online depository shows the study flow-diagram. Successful R_{int}, spirometry, body plethysmography and CO diffusion were obtained in respectively 1225, 1439, 969 and 715 children. From these measurements respectively 348, 397, 246 and 172 children met at least one of the exclusion criteria or had incomplete information on the exclusion criteria and were excluded. Baseline characteristics are shown in table 1. In the online depository age distributions are accessible in table 1.

Reference equations

The Gamma distribution fitted best for most outcome parameters. The BCCG distribution had the best fit for RV_{He}, RV_{He}/TLC_{He}, FEV_1/FVC and ERV_{pleth} and the normal distribution fitted best for $D_{L,CO}/VA$. A cubic spline approach for age and height did

Table 1. Baseline characteristics of children participating in different pulmonary function tests

		R_{int} N = 877	Spirometry N = 1042	Body plethysmography N = 723	CO diffusion / He dilution N = 543
Age (yr)	*median*	10.0	12.2	12.8	13.3
	*IQR**	6.9 – 13.4	8.0 – 14.0	10.3 – 14.6	11.8 – 15.0
	range	2.1 – 18.4	4.0 – 18.4	6.0 – 18.4	7.0 – 18.4
Height (cm)	*median*	142.3	153.5	160.0	164.1
	IQR	124.4 – 163.2	131.4 – 168.1	143.8 – 170.0	150.5 – 172.0
	range	84.1 – 193.0	102.4 – 193.0	112.0 – 193.0	121.7 – 193.0
Sex (male)	*n*	450	527	358	278
	%	51.3	50.6	49.5	51.2

* IQR = Interquartile range

not result in better fitting models. However, logarithmic transformation of age and/or height sometimes improved the fit. Q-statistics and 'worm plots' showed adequate fit of all final models [22].

Reference equations for all outcome measures are presented in Table 2a, 2b and 2c. Since most outcome measures are non-normally distributed, one cannot easily calculate the normal range. Therefore we constructed a file to compute predicted values, percentile scores and lower and upper limit of normal (LLN; 5th percentile and ULN; 95th percentile), accessible from the online supplement.

Mean

Most outcome measures were significantly related to Ln(height) (or height) and Ln(age) (or age) and sometimes their interaction had to be included (see tables 2a, 2b and 2c). V_T, $Raw_{0.5}$, $DL_{,CO}$ and FRC_{He} were significantly dependent on height only and not on age. In contrast, $RV_{He/}TLC_{He}$ was significantly dependent on age only and not on height. RV, RV/TLC, FRC_{pleth}, $Raw_{0,5}$, R_{int} and FEF values were independent of sex. An interaction between height and sex was included in the model for FVC.

Variability

The coefficient of variation (sigma) was constant for most outcome measures, except for ERV_{pleth} (height), $D_{L,CO}$ (height, age)and FRC_{He} (age, sex). For example, with increasing height the coefficient of variation of ERV_{pleth} decreases and the range of normality becomes smaller. The coefficients of variation are presented in table 2a, 2b and 2c.

Table 2. Reference equations for spirometry (n= 1042; age 4-18 yrs) (2a), body plethysmography (n= 723; age 6-18 yrs) (2b) and interrupter resistance (R_{int}) (n=877; age 2-18 yrs) (2b) and diffusion capacity for carbon monoxide ($D_{L,CO}$) and lung volumes measured with single breath helium dilution (n= 543; age 7-18 yrs) (2c).

2a)

Index	Mean (Mu)	Coefficient of variation (Sigma)
FEV_1 (l)	$e^{-1.74 + 0.016*H + 0.0017*A + 0.036*S}$	$e^{-2.19} = 0.11$
$FEV_{0.5}$ (l)	$e^{-1.81 + 0.015*H + 0.0020*A + 0.021*S}$	$e^{-2.08} = 0.13$
FVC (l)	$e^{-11.10 + 2.37*Ln(H) + 0.0016*A - 0.61*S + 0.13*Ln(H)*S}$	$e^{-2.19} = 0.11$
FEF_{75} (l/s)	$e^{36.05 - 7.47*Ln(H) - 9.57*Ln(A) + 1.98*Ln(H)*Ln(A)}$	$e^{-1.17} = 0.31$
FEF_{50} (l/s)	$e^{15.31 - 3.13*Ln(H) - 4.99*Ln(A) + 1.056*Ln(H)*Ln(A)}$	$e^{-1.46} = 0.23$
FEF_{25} (l/s)	$e^{-7.58 + 1.76*Ln(H) + 0.0024*A}$	$e^{-1.70} = 0.18$
FEF_{25-75} l/s)	$e^{22.28 - 4.61*Ln(H) - 6.59*Ln(A) + 1.39*Ln(H)*Ln(A)}$	$e^{-1.41} = 0.24$
PEF (l/s)	$e^{-7.98 + 1.85*Ln(H) + 0.0030*A + 0.036*S}$	$e^{-1.88} = 0.15$
FEV_1/FVC (%) ‡	$982.77 - 179.27*Ln(H) - 177.09*Ln(A) - 2.30*S + 35.57*Ln(H)*Ln(A)$	$e^{-2.22} = 0.11$
$FEV_{0.5}$/FVC (%)	$e^{-16.18 - 2.44*Ln(H) - 2.16*Ln(A) - 0.041*S + 0.44*Ln(H)*Ln(A)}$	$e^{-2.77} = 0.06$

H: Height (cm), A: Age (months), S: Sex (female= 0, male = 1).
‡ FEV1/FVC ratio for children aged 6-18 years (n=925)

Example: In a boy aged 12 year (144 months), height 160 cm, the predicted FEV_1 is:
FEV_1 = exp(-1.74 + 0.016*160 + 0.0017 * 144 + 0.036*1) = 3.01 L.

2b)

Index	Mean (Mu)	Coefficient of variation (Sigma)
RV_{pleth} (l)	$e^{4.76 - 0.99*Ln(H) - 0.11*A + 0.022*Ln(H)*A}$	$e^{-1.22} = 0.29$
TLC_{pleth} (l)	$e^{-5.30 + 1.29*Ln(H) - 0.040*A + 0.062*S + 0.0082*Ln(H)*A}$	$e^{-2.28} = 0.10$
ERV_{pleth} (l)	$e^{-4.28 + 0.019*H + 0.27*Ln(A) + 0.059*S}$	$e^{-0.24 - 0.0073*H}$
FRC_{pleth} (l)	$e^{-3.82 + 0.87*Ln(H) - 0.063*A + 0.046*S + 0.013*Ln(H)*A}$	$e^{-1.73} = 0.18$
IVC_{pleth} (l)	$e^{-1.65 + 0.016*H + 0.0016*A + 0.072*S}$	$e^{-2.19} = 0.11$
RV_{pleth}/TLC_{pleth}	$e^{7.87 - 0.056*H - 1.83*Ln(A) + 0.011*H*Ln(A)}$	$e^{-1.39} = 0.25$
VT (l)	$e^{-9.41 + 1.79*Ln(H) + 0.10*S}$	$e^{-1.22} = 0.30$
Raw_{TOT} (kPa·L^{-1}·s)	$e^{1.86 - 0.016*H - 0.0018*A}$	$e^{-1.39} = 0.25$
$Raw_{0.5}$ (kPa·L^{-1}·s)	$e^{1.52 - 0.018*H}$	$e^{-1.19} = 0.30$
R_{int} (kPa·L^{-1}·s)	$e^{-2.88 + 0.023*H + 0.82*Ln(A) - 0.0076*H*Ln(A)}$	$e^{-1.01} = 0.36$

H: Height (cm), A: Age (months), S: Sex (female= 0, male = 1).

2c)

Index	Mean (Mu)	Coefficient of variation (Sigma)
RV_{He} (l)	$e^{5.33 - 0.041*H - 1.57*Ln(A) + 0.011*H*Ln(A)}$	$e^{-1.42} = 0.24$
TLC_{He} (l)	$e^{24.67 + 4.91*Ln(H) - 7.16*Ln(A) + 0.059*S + 1.47*Ln(H)*Ln(A)}$	$e^{-2.27} = 0.10$
FRC_{He} (l)	$e^{-1.20 + 0.014*H}$	$e^{-6.20 + 0.033*H + 0.027*A - 0.00018*H*A}$
IVC_{He} (l)	$e^{-1.65 + 0.015*H + 0.0023*A + 0.072*S}$	$e^{-2.18} = 0.11$
RV_{He}/TLC_{He}	$e^{3.75 - 0.11*Ln(A)}$	$e^{-1.62} = 0.23$
VA (l)	$e^{25.27 - 5.04*Ln(H) - 7.29*Ln(A) + 0.061*S + 1.50*Ln(H)*Ln(A)}$	$e^{-2.24} = 0.11$
$D_{L,CO}$ (mmol/min/kPa)	$e^{34.80 - 6.89*Ln(H) - 8.66*Ln(A) + 0.10*S + 1.79*Ln(H)*Ln(A)}$	$e^{-2.38 + 0.0023*A + 0.75*S - 0.0052*A*S}$
$D_{L,CO}/V_a$ (mmol/min/kPa/l)	$2.37 - 0.0033*H + 0.072*S$	$e^{-1.45} = 0.23$

H: Height (cm), A: Age (months), S: Sex (female= 0, male = 1).

For calculation of percentile scores, lower (LLN) and upper limit of normal (ULN): use the file accessible from the online supplement.

Skewness

For most outcome measures the best fit was provided by the Gamma or normal distribution, in which skewness needs not be modelled separately. When the BCCG distribution fitted best, skewness (nu) has been modelled.

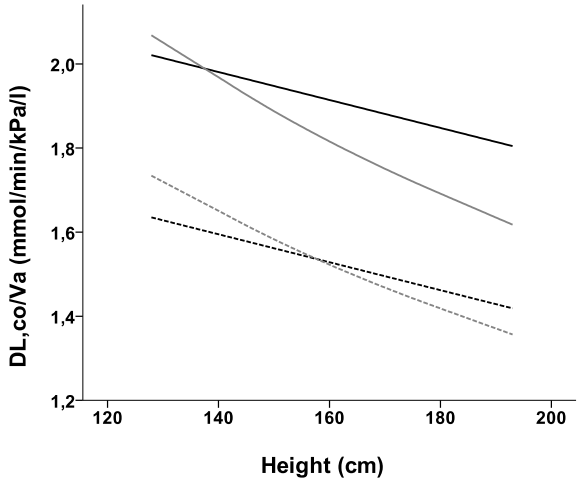
Comparison with previously published reference values

The following examples illustrate the developments in the new reference equations; mostly by comparing them with the most commonly used references.

Recently collected data

Figure 1 compares the new reference values for spirometry with those from Stanojevic, Zapletal and Hankinson [5,19,21]. The new predicted values for FEV_1 and FVC are systematically higher. When the new reference values for $D_{L,CO}/V_a$ are compared with those predicted by Stam [20], in young children the new $D_{L,CO}/V_a$ values are lower, whereas in older children the new predicted values are higher (figure 2). Figure 2 in the online depository shows the new reference values for Raw_{tot} plotted against height and compared with those predicted by Zapletal [21]. The new predicted values for Raw_{tot} are systemically higher.

Figure 2. Diffusion capacity of the lung for carbon monoxide (CO) in boys (n=278), plotted against height. Lines represent predicted values based on new reference equation (black) and predicted values based on the reference equation by Stam et al.[20] (grey). Dotted lines represent lower limit of normal (5th percentile).



No extrapolation beyond the intended age range

In figure 3 the mean R_{int} values as well as the ULN are shown and compared to those recently published by Merkus [2]. In the younger and smaller children the mean predicted values are rather different but the ULN values are more similar. However, when the Merkus equations are extrapolated beyond the intended age range (13 yrs), the values are higher than the new predicted values. If those reference values were used in children above the age of 13, fewer children would be classified as having an abnormal R_{int} value. The effect of extrapolation beyond the intended height/age range can also be seen when predicted FEV_1 values from Hankinson are compared with the new values in children younger than eight years of age.

Inclusion of both height and age as determinants

Unlike most previously published reference equations, age was entered in the models as possible determinant. Most parameters were related to Ln(age) (or age) and therefore these parameters were higher for children with comparable height but older age (see figure 3 in online depository).

In figure 4 the newly predicted reference values for the FEV_1/FVC ratio are compared with those from Stanojevic, Zapletal and Hankinson[5,19,21], for girls and boys separately. Inclusion of height and age as well as their interaction resulted in a decrease in FEV_1/FVC ratio until the beginning of puberty, followed by a small increase.

Figure 3. Interrupter resistance (R_{int}) plotted against height for 427 girls (3a) and 450 boys (3b). Lines represent predicted values based on new reference equation (––––) and predicted values based on the reference equation by Merkus et al.[2] (––––). Dotted lines represent upper limit of normal (95th percentile).

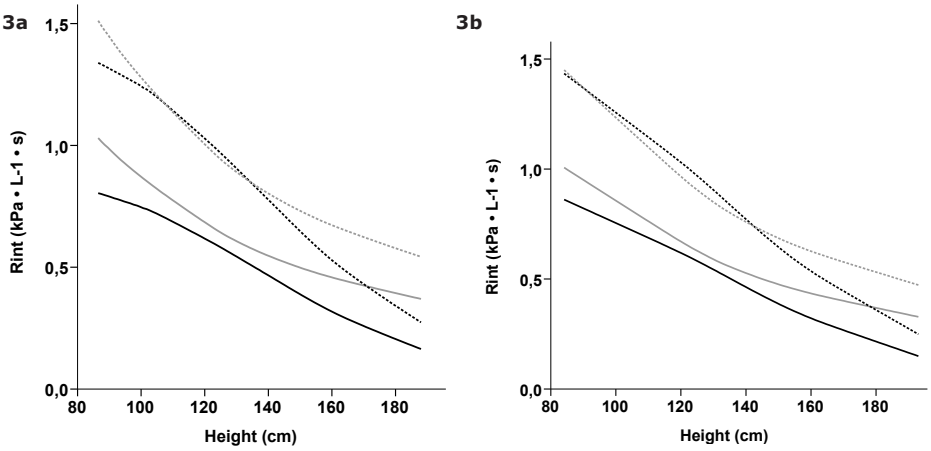
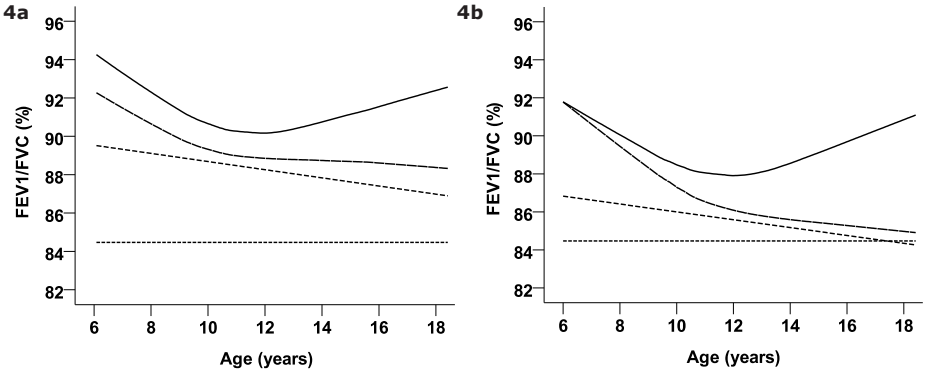


Figure 4. FEV1/FVC ratio (%) plotted against age for 454 girls (4a) and 471 boys (4b). Lines represent predicted values based on new reference equation (––––) and previous reference equations by Zapletal et al. [21](·····), Hankinson et al. [19] (- - - -) and Stanojevic et al. [5](— — —).



Range of normality

In figure 5, z-scores based on Zapletal's reference equation for TLC_{pleth} and RV_{pleth} [21] are plotted against height; the plot includes the LLN below which 5% of observations should fall. There are too few observations outside the normal range. Figure 4 in the online depository shows a similar phenomenon for FEV_1, as well as too many FEF_{75} measurements below the LLN. There is an age-related trend in average z-scores with a nadir during puberty. The percentage of children in whom a spirometric index fell below the LLN according to Zapletal is shown in figure 5 in the online depository; for

Figure 5. Z-scores of TLC_{pleth} (5a) and RV_{pleth} (5b) based on the reference equations by Zapletal et al.[21] applied to the female study population (365 girls aged 6-18). Too few measurements are below the lower limit of normal (Z-score = -1.645; 5th percentile) or above the upper limit of normal (Z-score = 1.645; 95th percentile).

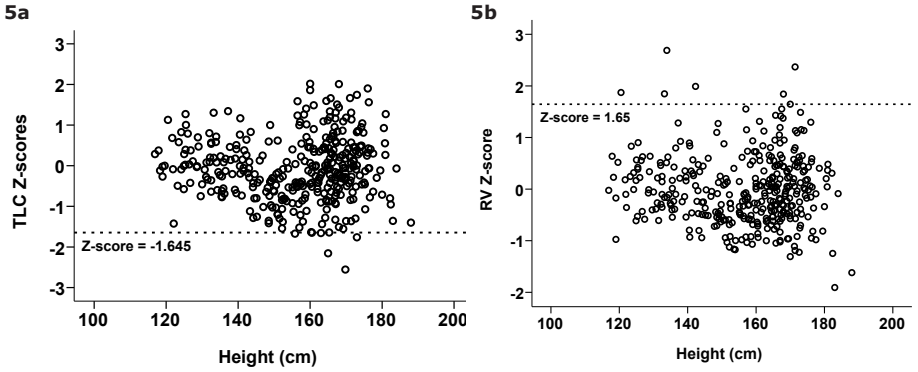
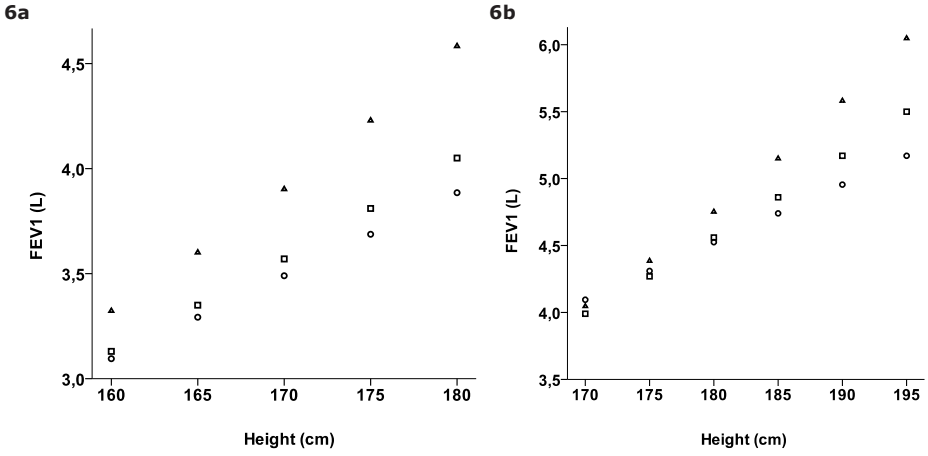


Figure 6. FEV_1 values predicted by the new reference equation (▲) for subjects aged 18 years with different heights, for girls (6a) and boys (6b) separately. These new reference equations do not smoothly connect with ECSC/ERS [23] reference equations for adults (○) and the all-age reference equations by Stanojevic [5] (□).



instance in only 1.2% of the girls did the FEV_1 fall below the LLN, compared to 28.1% for FEF_{75} in boys.

Transition from paediatric to adult reference values

Figure 6 shows that there is no smooth connection between these new reference equations for children and the currently used reference equations in adults [5,23].

Discussion

This study provides new reference values for all most commonly used pulmonary function tests in children aged 2-18 years, based on newly collected data and analyzed using the GAMLSS method. It gives an accurate prediction of pulmonary function of the current Dutch paediatric population, which we consider applicable to other Caucasians.

We consider these new reference values more representative for today's paediatric population for a number of reasons. Firstly, we provide reference equations based on newly collected data, measured between 2004 and 2008. The currently recommended[6] reference equations for lung volumes measured by helium dilution[24] and bodyplethysmography[21] and the most commonly used reference values for spirometry[5,19,21] were obtained between 1960 and 1990. Due to an improved nutrition and the generally better health, the age-height relationship will have changed over the past decades[25]. Furthermore the age at onset of puberty decreased[26]. These secular trends in body growth probably have affected pulmonary function parameters. This might explain why, for instance, the new reference values for spirometry generally turn out higher than previously published spirometric reference values. Additionally, to some degree secular trends in lung function could be explained by modifications in measurement protocols over the past decades [27]. The presented reference equations have the benefit that all measurements were performed in accordance with the latest ATS/ERS statements and using identical equipment in every subject.

Secondly, these new reference values cover the whole paediatric age range from 2 to 18 years. When reference values are applied in children younger than the reference population, it is common practice to extrapolate available reference equations beyond the intended height and age. This is discouraged because it may lead to age/height dependent over- or underestimation of pulmonary function. When using the presented reference equations one should be aware of the fact that the minimal age differed for the different techniques. As an example, for R_{int} measurements, considered a reasonable alternative for FEV_1 [28] reference values for children aged 2-13 have been published[2], but reference values for Caucasian children aged 13-18 were lacking. Since the relationship between height and R_{int} is not constant, the reference equation based on children aged 3-13 years should not be extrapolated to older (and taller) children, because too few children would then be classified as having an abnormal R_{int} value (see figure 3).

Additionally, since spirometric measurements are nowadays feasible in younger age groups, data were also collected in children aged 4-6 years. The present study confirms the finding by Stanojevic et al.[5] that an underestimation of FEV_1 exists when

extrapolating Hankinson's prediction equations [19] below the valid age range (see figure 1).

Thirdly, in the new reference equations age was sometimes included as a determinant (see figure 3 in the online depository). Quanjer et al. first demonstrated that this results in better fitting reference equations [4], and this was recently corroborated by Stanojevic et al. [5]. During puberty lung growth lags behind the phase of rapid increase in height, resulting in a period of relatively low pulmonary function for height [29], as shown in figure 4 in the online depository. Failure to include age among explanatory variables leads to an age-related bias in most pulmonary function measures. This age-related trend was recently demonstrated in different data sets [30]. The most commonly used reference equations in the Netherlands [21] are based on height only, and fail to accurately describe the complex changes in pulmonary function during puberty. The new reference equations for the FEV_1/FVC ratio, including both height and age as well as their interaction, showed a fall until the start of puberty, followed by a rise in FEV_1/FVC, as recently shown by Quanjer et al.[31].

Another advantage of these new reference equations is the fact that sex was modelled as one of the possible determinants, instead of modelling separate equations for boys and girls as some studies did. This resulted, for example, in sex-independent equations for FEF values, which means that the association between FEF values and height, age and the height/age interaction is similar for both sexes. We suggest that the existing differences in FEF values between boys and girls can be explained by differences in height and the height/age interaction.

Furthermore, the new reference equations for CO diffusion and lung volumes measured with bodyplethysmography and single breath He dilution are based on relatively large samples of children, compared to previously published reference equations [20,21,24]. Therefore, the mean predicted values as well as the ULN and LLN could be calculated more accurately.

Additionally, since these new reference values are representative for the current paediatric population, thus resulting in a reliable range of normality. A reliable range of normality is a prerequisite for correct interpretation of individual test results. For example, less than five percent of the children have a TLC_{pleth} value below the LLN if the reference equation of Zapletal is used in the current population. This means that possible pathology could be overlooked. On the other hand, some outcome measures are overestimated by previously published reference equations, resulting in a too high lower limit of normal. Consequently, too many children could accidentally be misclassified as having an abnormal pulmonary function. Furthermore, we evaluated which distribution fitted best for each outcome measure, since pulmonary function outcome measures are often non-normally distributed. Taking the skewness of the

data into account, the correct calculation of the percentile score of an individual test result is possible.

In contrast to the study by Stanojevic et al, the coefficient of variation was constant for most parameters and was not higher in the youngest children. Young children have more difficulties performing a forced flow/volume measurement than older children, and this could result in more variation in the outcome measures. We used the same acceptability criteria for all children, possibly resulting in lower success rates in the youngest children. However, this could lead to a relatively small coefficient of variation compared to studies using less strict acceptability criteria in the youngest children. Furthermore, if the sample of children included per age stratum is small, the predicted normal range for that age group could also coincidently be too wide. Although we included a similar number of children younger than 8, it could be possible that we included a larger number of 4 and 5 year old children than were included in the study by Stanojevic et al, resulting in a more precise estimate of the coefficient of variation.

The present study has some limitations. Obviously, the results are not generalizable to non-Caucasian children, since ethnic differences in lung function exist [10]. However, one could argue whether these reference values could also be applied to non-European Caucasian children, since we included only children with at least one European parent. Although children from Northern European countries become taller than WHO growth charts predictions [32], the relationship between height/age and lung function could be similar in all Caucasians. There is insufficient evidence to guarantee that these new reference equations are applicable to all European and non-European Caucasians. Furthermore, the use of different equipment in other laboratories could reduce the generalizability. However, the use of a range of equipment in collated data studies will add extra (inter-equipment) variability to the reference values. This means that, although those studies generate more generalizable equations, those reference values will have wider ranges of normality.

Since generalizability to other populations and centres can never be guaranteed, centres are encouraged to validate the new reference values in their own population to test for systemic bias.

Another limitation of the present study is the fact that measurements were merely obtained in children. These new reference equations do not connect smoothly with the used reference equations for adults (figure 6) and therefore the need of collecting new data in adults, especially covering the adolescent and young adult period, persists.

As this is a cross-sectional study, reference ranges are based on cross-sectional samples, and no data regarding either short or longer-term repeatability of spirometry are available. However, longitudinal studies to generate reference values are hardly feasible.

Since specific ATS/ERS statements on paediatric pulmonary function testing do not exist (except for R_{int} measurements and spirometry in preschoolers), we used adult criteria for most tests. We sometimes made small adjustments to the protocols, because it was not always feasible to meet those criteria. The used criteria for body plethysmography differed from the criteria in adults, by occasionally accepting two instead of three FRC_{pleth} measurements that agreed within 5%. For spirometry we used the earlier published criteria [33] for all children, although different criteria for preschoolers have been published during the study period [14]. If we would have used these new criteria, this could possibly have resulted in more successful spirometry measurements in preschoolers.

Another limitation of this study is that the lung volumes measured with the He dilution technique were obtained using the single breath method instead of multiple breath method. The reader should realize that these reference values should not be used for volumes measured with the multiple breath He dilution technique.

In conclusion, we present new paediatric reference equations for pulmonary function, based on recently collected data that comply with recent quality criteria. These new reference values provide accurate estimates of the range of normality for most commonly used pulmonary function indices, resulting in less underdiagnosis and overdiagnosis of pulmonary diseases in today's paediatric population.

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Online supplement

Methods

The cross-sectional "Utrecht Pulmonary Function Reference Data Study" was performed by the Department of Paediatric Pulmonology of the University Medical Center Utrecht, the Netherlands, and approved by the local Medical Ethics Committee. Data were collected by experienced paediatric pulmonary function technicians, between January 2004 and April 2009 at ten randomly selected primary and secondary schools in the Provinces of Utrecht, Zuid-Holland and Gelderland. R_{int} measurements were also performed in 4 kindergartens.

Study population:

Children aged 2-18 were eligible. Informed consent was obtained from subjects and/or parents. Exclusion criteria were: doctor's diagnosis of asthma (ever), current use of asthma medication, wheezing in previous 12 months, cystic fibrosis, past thoracic surgery, neuromuscular disease, current active smoking, birth weight < 2000 gram or two non-European parents. Measurements were postponed for at least 6 weeks in children with signs of a respiratory tract infection.

Measurements

Measurements were performed in a mobile pulmonary function lab. Equipment was calibrated upon arrival at each location. Test order was: R_{int}, body plethysmography, spirometry and carbon monoxide (CO) diffusion/helium (He) dilution. Except for R_{int}, all measurements were BTPS corrected. Tests were performed in sitting position wearing a nose clip. Only measurements that complied ATS/ERS guidelines (and updated versions) [1-6] were included in the study.

Interrupter resistance (R_{int})

Expiratory R_{int} measurements were carried out in children aged 2-18, using MicroRint (Micro Medical Limited, Kent, UK). This portable device contains a pneumotachometer, a flow interruption valve and a pressure transducer to measure mouth pressure post-occlusion. R_{int} measurements were in agreement with the latest ATS/ERS guideline on interrupter technique [2]. Measurements were performed in a sitting position and the child was diverted to ensure quiet tidal breathing. Subjects wore a nose clip and were instructed to seal their lips around the mouthpiece with bacterial filter to prevent air leakage. The technicians supported the cheeks with their hands to reduce the change

in upper airway compliance. Interruptions of 100 ms were made at peak expiratory flow with a random frequency, with the valve closing within 10 ms.
The MicroRint software automatically rejected measurements that showed an artefact on the pressure curve. At first ten successful interruptions were performed. Additionally measurements that showed tachypnoea or irregular breathing, vocalization, or hyperextension or flexion of the neck were manually discarded as were tracings with a horizontal or declining pressure signal suggesting air leakage around the mouthpiece. R_{int} values were calculated using the two-point linear fit back extrapolation technique to t=15ms [7]. A minimum of five out of ten interruptions was required to calculate the median R_{int} value.

Body plethysmography

Lung volume and airway resistance measurements were acquired in children aged 6-18 using a variable pressure plethysmograph (MasterScreen Body, Cardinal Health, Hoechberg, Germany).
Measurements were carried out with the child sitting comfortably in the chamber, without having to flex or extend the neck. After closing the door, time was allowed for the thermal transients to stabilize and for the child to relax. The child was asked to breathe quietly through the mouthpiece until a stable end-expiratory baseline presenting functional residual capacity (FRC_{pleth}) was established. Airway resistance was measured 5 times by drawing a line connecting the intercepts of the flow/volume loop at inspiratory and expiratory flows of 0.5 L/s ($Raw_{0.5}$). Additionally the airway was occluded by a shutter at FRC_{pleth} and the child was instructed to perform a series of 3-5 gentle panting manoeuvres at a frequency of about 0.5 Hz. The manoeuvres had to result in almost superimposable straight lines in the $\Delta P/ \Delta V$ plot, with only a small separation by thermal drift. After opening of the shutter the child was instructed to inspire to total lung capacity (TLC) and subsequently perform a slow expiratory vital capacity manoeuvre followed by a slow inspiratory vital capacity manoeuvre.
The aim was to obtain 3 FRC_{pleth} technician-accepted measurements that agreed within 5% (one of the criteria in the latest adult guideline [6]), but if unfeasible, 2 comparable measurements were considered sufficient. Out of these measurements we computed the mean FRC_{pleth}, the mean expiratory residual volume (ERV) and the highest inspiratory vital capacity (IVC). Residual volume (RV) and TLC were calculated from these values as follows:

$$RV = FRC_{pleth} - ERV$$
$$TLC = RV + IVC$$

Mean tidal volume (VT) and $Raw_{0.5}$ were computed from 5 measurements.

Spirometry

Spirometry was performed in children aged 4-18 using a heated Lilly head pneumotachometer system (Cardinal Health, Kleve, Germany). After full inspiration the children performed a maximally forced and full expiration. At least three technician-accepted flow-volume curves out of maximally eight attempts were obtained. The largest forced expiratory volume in 0.5 or 1 second ($FEV_{0.5}$ or FEV_1), forced vital capacity (FVC) and peak expiratory flow (PEF) were selected. The curve with the highest sum of FEV_1 and FVC was selected for analysis of maximal expiratory flows when 25%, 50%, 75% of FVC is expired (FEF_{25}, FEF_{50}, FEF_{75}) and maximal mid-expiratory flow (FEF_{25-75}).

Single breath CO diffusion and He dilution

Diffusion capacity of the lung was determined using the single breath CO diffusion technique ($D_{L,CO}$) and lung volumes (alveolar volume (VA), RV, TLC, FRC, IVC) were assessed using single breath He dilution. Tests were performed with the MasterScreen Diffusion (Cardinal Health, Hoechberg, Germany) in children with a FVC (measured during spirometry) of 1.5 L or above. The child was instructed to breath quietly through the mouthpiece. Subsequently the child was asked to exhale to RV. At RV the mouthpiece was connected to the test gas (0.28% CO, 9.5% He, ambient air) and the subject inhaled rapidly to TLC. After ten seconds breath-hold the child exhaled unforced but fast and the expired volume was divided into an initial dead space washout and an alveolar sample that was subsequently analyzed. At least two $D_{L,CO}$ measurements with a maximal difference of 10% were obtained with four minutes intervals and mean $D_{L,CO}$ was recorded. For $D_{L,CO}$ values we made the assumption of normal haemoglobin concentration in the study population.

Data analysis

Data were analyzed using the GAMLSS-package (Mikis Stasinopoulos and Bob Rigby with contributions from Calliope Akantziliotou (2008). GAMLSS: Generalized Additive Models for Location Scale and Shape. Version 1.9-4. http://www.gamlss.com) [8] in the statistical program R (R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.). GAMLSS is a framework for fitting regression type models that allows many distributions of the outcome variable. GAMLSS models the location (median or mean, *mu*), variability (*sigma*) and dependent on the distribution chosen also the skewness (*nu*) and kurtosis (*tau*). The following explanatory variables were considered: age, height and sex and their two-way interaction terms. The Schwartz Bayesian criterion (SBC) was used to select the simplest model with adequate fit.

Modelling started with comparing three eligible distributions: normal, Box-Cox-Cole-Green (BCCG) and Gamma. After selection of the distribution, we assessed whether a logarithmic transformation of the outcome variable was required. It was determined whether the effects of age and height should be modelled linearly, logarithmically transformed and whether a cubic spline improved the fit. An SBC guided backward analysis was performed to eliminate non-significant explanatory variables. Q-statistics and 'worm plots' were used to assess the goodness of fit of the final models [9]. Modelled reference equations were compared with those most commonly used in Europe and North-America [10-13].

Results

Study population

Figure 1 shows the study flow-diagram. In table 1 age distributions of the total study population are accessible.

Figure 1. Flow chart of study population.

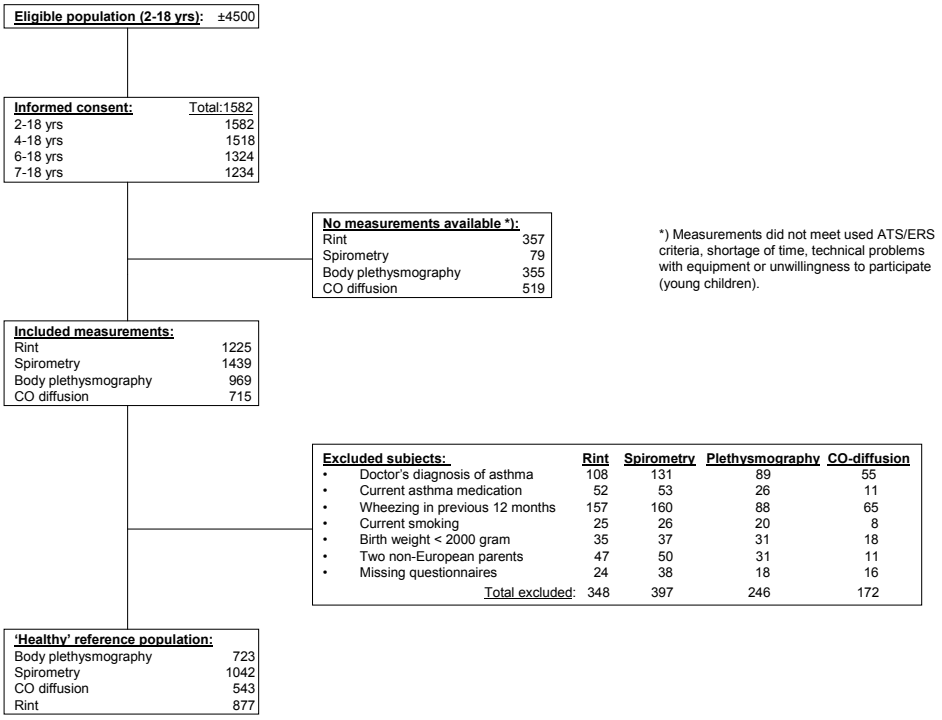


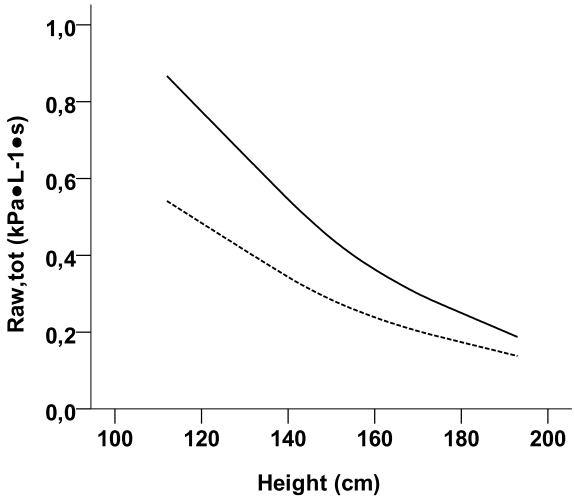
Table 1. Age distribution of reference population.

Age (in years)	R_{int} N = 877	Spirometry N = 1042	Body plethysmography N = 723	CO diffusion / He dilution N = 543
2	12	-	-	-
3	19	-	-	-
4	55	51	-	-
5	76	66	-	-
6	60	56	24	-
7	87	87	43	23
8	72	68	50	28
9	53	54	43	24
10	71	68	58	36
11	47	52	44	33
12	69	141	122	95
13	84	134	118	102
14	81	98	73	66
15	50	84	74	63
16	19	48	45	41
17	14	24	19	22
18	8	11	10	10

Evaluation of the new reference values

Figure 2 shows the new reference values for Raw_{tot} plotted against height and compared with those predicted by Zapletal [13]. The new predicted values for Raw_{tot} are systemically higher.

Figure 2. Raw_{tot} plotted against height. Lines represent predicted values based on new reference equation (––––) and predicted values based on the reference equation by Zapletal [13] (- - - - -) (n=723).



Most parameters were related to ln(age (or age) and therefore these parameters were higher for children with comparable height but older age (see figure 3).
Figure 4 shows that too few FEV_1 measurements are below the lower limit of normal (LLN; 5th percentile), whereas too many FEF_{75} measurements are below the LLN.

Figure 3. Reference values for FEV_1 against height, for different age categories, for boys (3a) and girls (3b).
⋯⋯ = 4-7 yr, - - - = 7-10 yr, – – – = 10-13 yr, ——— = 13-16 yr, –––– = 16-18 yrs

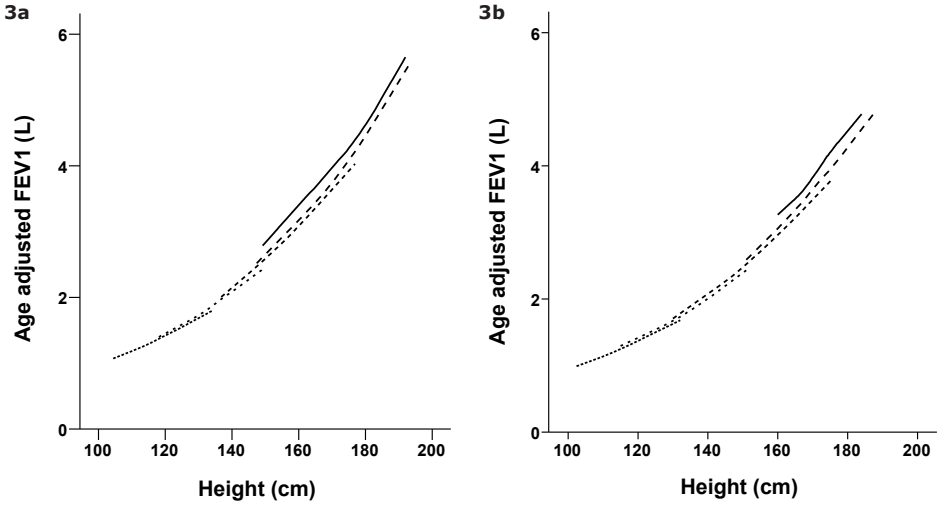


Figure 4. Scatter plot of z-scores based on Zapletal's reference values [13] of FEV_1 (4a) and FEF_{75} (4b) plotted against height. Bold line represents average z-scores. Dashed line represents the lower limit of normal (LLN) set at a z-score of −1.645 (5th percentile). Too few children have a FEV_1 z-score below LLN (2.2%) and too many have a FEF_{75} z-score below LLN (24.4%).

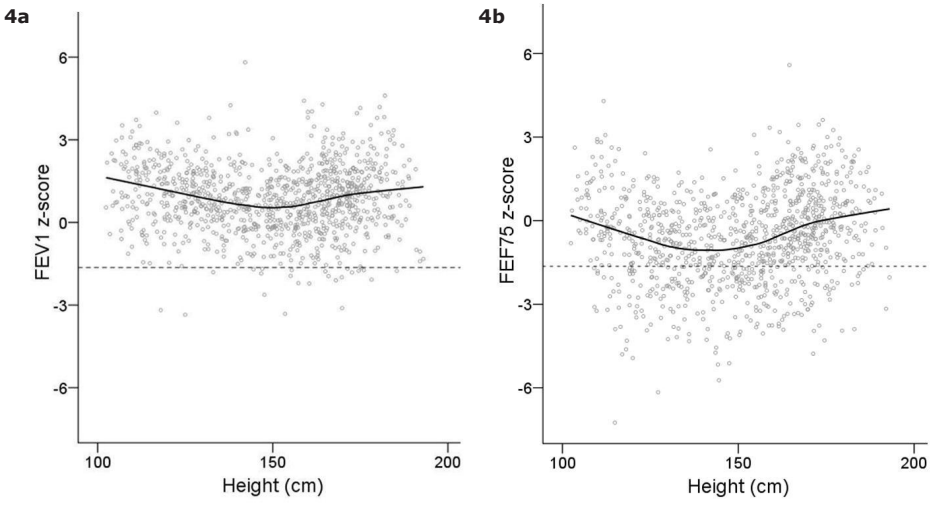
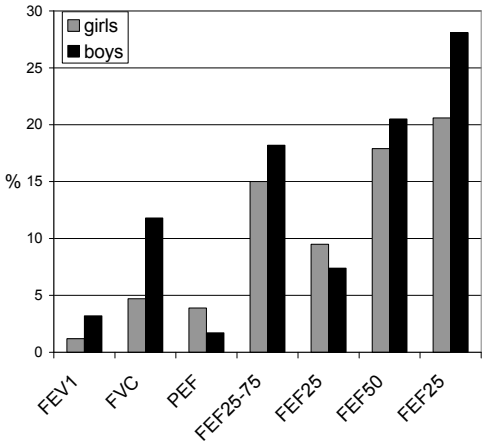


Figure 5. Percentage of children with a z-score below the Lower Limit of Normal (z-score -1.645) based on Zapletal's reference values [13], shown for several spirometric indices, and for girls and boys separately.



There is an age-related trend in average z-scores with a nadir during puberty. The percentage of children in whom a spirometric index fell below the LLN according to Zapletal is shown in figure 5; for instance in only 1.2% of the girls did the FEV_1 fall below the LLN, compared to 28.1% for FEF_{75} in boys.

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CHAPTER 6B

The effect of tobacco smoke exposure on lung function in a healthy reference population

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Introduction

In the study on lung function reference values, we choose not to exclude children who were exposed to environmental tobacco smoke (ETS). However, since ETS exposure has adverse effects on peripheral airway function in infancy[1], school age[2] and adolescence[3], the inclusion of ETS exposed children in the reference population could have resulted in somewhat reduced reference values for peripheral airway function. Using spirometry as an example, we investigated whether ETS exposed otherwise healthy children have reduced lung function compared to non-exposed children.

Methods

The influence of ETS exposure on lung function was investigated in the healthy population used to generate reference values for spirometry. For detailed information see chapter 6A.

Environmental tobacco smoke exposure

Questions on ETS exposure related to domestic parental smoking during childhood. Since a precise estimate of past ETS exposure could easily be biased by recall it was not quantified but dichotomized. When one or both parents reported to smoke or have smoked now and/or in the past, the child was categorized as "ever ETS exposed". Children whose parents both reported never to have smoked in the home were considered "never ETS exposed".

Statistical analysis

Height, age and sex of "ever ETS exposed" and "never ETS exposed" children were compared using Mann-Whitney-U-tests and chi-square-test. Differences in spirometric measures between "ever ETS exposed" and "never ETS exposed" children were analyzed using general linear models, including height, age and sex.

Results

785 and 257 children were classified as "never ETS exposed" and "ever ETS exposed" respectively. Baseline characteristics are presented in Table 1.

There were no significant differences in age and height between 'never ETS exposed' children and 'ever ETS exposed children' (Mann-Whitney U test: p=0.058 and p=0.163 respectively). There was also no difference in sex distribution (chi-square test: p=0.883).

Table 1. Baseline characteristics comparing "never ETS exposed" and "ever ETS exposed" subjects.

		Never ETS exposed N = 785 (75%)	Ever ETS exposed N = 257 (25%)	p-value
Age (yr)	*median (IQR)*	12.1 (7.7-14.0)	12.3 (9.3-14.0)	0.058
	range	4.1-18.4	4.0-18.2	
Height (cm)	*median (IQR)*	152.4 (130.0-168.0)	155.1 (136.4-168.5)	0.163
	range	102.4-193.0	105.3-189.0	
Sex (male)	*n* (%)	396 (50.4%)	131 (51.0%)	0.883

ETS = Environmental tobacco smoke
IQR = Interquartile range

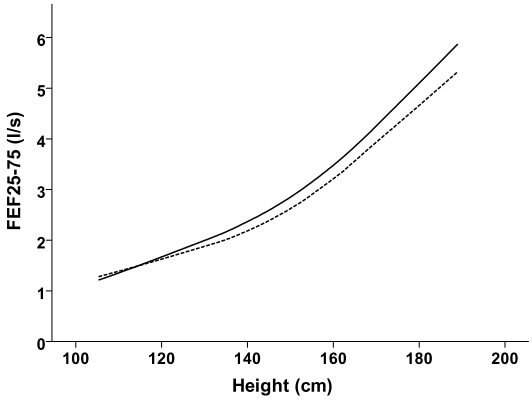
ETS exposure and lung function

Table 2 shows the differences in lung function between "ever ETS exposed" and "never ETS exposed children". There were significant reductions of FEF_{75}, FEF_{50}, FEF_{25} and FEF_{25-75} values in "ever ETS exposed" children (minus 9.4%, 7.4%, 5.4% and 8.1% respectively ($p \leq 0.001$)) and also small but significant reductions in FEV_1, $FEV_{0.5}$ and PEF. Figure 1 shows the differences between mean FEF_{25-75} values measured in 'ever ETS exposed' children and age-adjusted predicted FEF_{25-75} values based on the new reference equations. The predicted values are on average "too high" for children exposed to ETS.

Table 2. Comparison of mean spirometric values between children categorized as "never ETS exposed" (n=785) and children categorized as "ever ETS exposed" (n= 257), adjusted for age, height and sex.

	"never ETS exposed"	"ever ETS exposed"	Mean difference	95% CI	p-value
FEV_1 (l)	2.748	2.658	-3.3%	-5.3% – -1.2%	0.002
$FEV_{0.5}$ (l)	2.115	2.027	-4.2%	-6.4% – -2.0%	<0.001
FVC (l)	3.057	3.018	-1.3%	-3.2% – 0.7%	0.208
FEF_{25} (l/s)	5.002	4.732	-5.4%	-8.2% – -2.6%	<0.001
FEF_{50} (l/s)	3.451	3.194	-7.4%	-11.0% – -3.9%	<0.001
FEF_{75} (l/s)	1.708	1.547	-9.4%	-14.3% – -4.6%	<0.001
FEF_{25-75} (l/s)	5.993	5.771	-8.1%	-12.0% – -4.2%	<0.001
PEF (l/s)	3.158	2.902	-3.7%	-6.1% – -1.3%	0.003

Figure 1. Mean FEF_{25-75} values measured in 'ever ETS exposed' children compared with age-adjusted predicted FEF_{25-75} values versus height.
——— = Age adjusted predicted FEF_{25-75} values
------- = Measured FEF_{25-75} values in 'ever ETS exposed' children



Discussion

This study shows that ETS exposure in healthy children without any history of pulmonary or airway disease nor recent pulmonary airway symptoms, results in a reduction of parameters of peripheral airway function.
We choose not to exclude ETS exposed children from our reference population. Since we showed that these children on average have a reduction of forced expiratory flows and volumes, one may argue about the justification of this choice. In our opinion, there are at least two reasons that justify the inclusion of the ETS exposed children. First, ETS exposure as measured in this study resulted in about 8% reduction of peripheral airway measures. Since 25% of the children were exposed to ETS ever in life, including these children in the reference population would have resulted in approximately 2% reduction of the reference values. Although this would have been a significant reduction, it is so small that it would hardly have implications for diagnosing lung disease, specifically because measures of peripheral airway function showed large variability. Second, although it seems reasonable to exclude ETS exposed children in order to create a "healthy" reference population, ETS exposure is only one of several (unknown) exposures causing a reduction in lung function. For example, air pollution reduces lung function as well[4]. If we would have excluded all children exposed to all possible deteriorating influences, only extremely rare "healthy" cases would have remained and such a "healthy" reference population would not be generalizable to the paediatric population visiting a lung function laboratory.
One could also question whether ETS exposure should have been analyzed as a determinant of lung function measures. Although it might be interesting to identify

to which extent lung function reduction is caused by ETS and which part is caused by a lung disease, analyzing ETS exposure as a determinant of lung function cannot be recommended. In order to be utilized as a determinant in generating reference values, quantification of the exposure should be precise, such as the measurement of height and age. It is not feasible to quantify ETS exposure properly when generating reference values or during clinical lung function testing. Therefore, ETS exposure cannot serve as one of the regular determinants of lung function.

Since we showed that ETS exposure results in decreased lung function in otherwise healthy children, ETS exposure should always be part of history taking, in order to interpret lung function appropriately. Moreover, parents and caregivers should be discouraged to smoke in the nearness of their children, especially those suffering from lung diseases.

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CHAPTER 7

Evaluation of interrupter resistance in methacholine challenge testing in children

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Abstract

Introduction

Bronchial hyperresponsiveness is a key feature of asthma and is assessed using bronchial provocation tests. The primary outcome in such tests (a 20% decrease in forced expiratory volume in 1 second (FEV_1)) is difficult to measure in young patients. This study evaluated the sensitivity and specificity of the interrupter resistance (R_{int}) technique, which does not require active patient participation, by comparing it to the primary outcome measure.

Methods

Methacholine challenge tests were performed in children with a history of moderate asthma and bronchial hyperresponsiveness. Mean and individual changes in R_{int} and FEV_1 were studied. A receiver operating characteristic (ROC) curve was used to describe sensitivity and specificity of R_{int}.

Results

Seventy-three children (median age: 9.2 yrs; range: 6.3- 13.4 yrs) participated. There was a significant ($p< 0.01$) increase in mean R_{int} with increasing methacholine doses. However, individual changes of R_{int} showed large fluctuations. There was great overlap in change of R_{int} between children who did and did not reach the FEV_1 endpoint. A ROC-curve showed an area under the curve of 0.65.

Conclusion

Because of low sensitivity and specificity, the use of R_{int} to diagnose bronchial hyperresponsiveness in individual patients seems limited.

Introduction

According to GINA guidelines bronchial hyperresponsiveness (BHR) is a key feature of asthma [1]. To objectify BHR, forced flow volume manoeuvres are required to measure the forced expiratory volume in one second (FEV_1), as primary outcome measure in bronchial challenge testing [2]. Because of the difficulty of obtaining forced lung function measurements in early childhood the diagnosis of asthma in this age group is challenging and complicated. Several studies have advocated measurement of the interrupter resistance (R_{int}) as an alternative to quantify airway obstruction in preschool children and in children with developmental delay [3;4]. The interrupter technique is non-invasive and can be obtained during tidal breathing [5]. R_{int} can be measured in children from two years of age as it requires minimal cooperation [6]. The R_{int} technique has been compared to body plethysmography [7] and transcutaneous partial pressure of oxygen measurements [7-10] during methacholine challenge tests, but most studies made no comparison with the outcome measure FEV_1. The one study that compared R_{int} with FEV_1 during methacholine challenge testing reported a mean increase in R_{int} with increasing doses of methacholine [11]. However, this study did not present the changes in R_{int} for individual patients nor how these related to individual changes in FEV_1. The ability of R_{int} to detect methacholine induced bronchoconstriction in individual patients, as measured by a 20% decrease in FEV_1, has never been studied.

The latest ATS/ERS statement on pulmonary function testing in preschool children recommended the need to determine the role of R_{int} in bronchial challenge tests [5]. The current study evaluated the sensitivity and specificity of R_{int} to detect airflow obstruction during methacholine challenge tests in asthmatic children by comparing measurements with FEV_1 as the gold standard.

Methods

This study was a substudy of the multicentre COMBO-study in children between 6 and 16 years with moderate asthma (see online supplement) [12]. Nine out of 19 hospitals performed additional R_{int} measurements during methacholine challenge tests. The children from these centres were included in this substudy. It was approved by the local medical ethical committees and written informed consent was obtained from the parents (and the child if ≥12 years) before start of the study. For additional information see online supplement.

Study protocol

Methacholine provocation tests were performed twice (at start of study and after 26 weeks) measuring R_{int} and FEV_1 after each methacholine dose. All children abstained from long-acting β2 agonists and inhaled steroids for 12 hours before the first methacholine provocation test (and 36 hours before the second methacholine provocation test), and from short acting β2 agonists for 6 hours before provocation testing. To prevent possible, unwanted physiologic effects of deep inhalation on R_{int}, R_{int} was measured prior to FEV_1. Pulmonary function tests were performed to conform with latest ATS/ERS guidelines [2;5;13] and briefly described below. The complete methodology is described in the online supplement.

Methacholine challenge testing

Methacholine challenge tests were performed using the dosimeter method [2;14] with a nebulizer (model 646, DeVilbiss Healthcare, Somerset, USA) attached to a dosimeter (Rosenthal French dosimeter, PDS Instrumentation, Louisville CO, USA). Methacholine chloride was administered in doubling doses and provocation was continued after at least five minutes and until the dose at which at least 20% fall in FEV_1 (PD_{20}) or the maximum dose was reached.

Interrupter resistance

Airway resistance measurements were carried out using the MicroRint (Micro Medical Limited, Kent, UK). A bacterial filter was used (Spirosafe bacterial filter, Micro Medical, Kent, UK). Reference values of Merkus et al. [15] were used.

Spirometry

Maximal expiratory flow-volume measurements were performed with standardized equipment, using reference values of Stanojevic [16].

Analysis

Changes in FEV_1 and R_{int} were analyzed as percentages change from baseline. Change in R_{int} was also assessed as change in percentage predicted and change in z-score, computed from the latest R_{int} reference values [15]. Wilcoxon Signed Ranks test was used to investigate whether there was a significant increase in mean change from baseline of R_{int} at each dose step. To quantify the variability of FEV_1 and R_{int} within individuals between the various dosesteps, coefficients of variation (CV) were calculated (see online supplement for calculation). The resulting individual paired CV's of FEV_1 and R_{int} were compared using Wilcoxon's test. Comparison of mean CV's between centers was done using the Kruskal-Wallis test. The association between changes in FEV_1 and changes in R_{int} at PD_{20} was analysed using Spearman's correlation (r_S). At each

separate dose step Mann Whitney tests were computed to compare the difference in changes from baseline between subjects who did and who did not reach the PD_{20} endpoint at the dose step. Cox regression, with change from baseline of R_{int} as time-dependent variable, was used to analyse whether these changes were predictive for having reached the PD_{20} endpoint at the subsequent dose steps. The sensitivity and specificity of R_{int} in relation to having reached FEV_1-based PD_{20} was described by receiver operating characteristic (ROC) curves. The area under the curve (AUC) shows whether the change in R_{int} can correctly classify those who do and do not reach the PD_{20} endpoint. We used the coefficient of repeatability calculated by Beydon et al. (a 32.1% increase in R_{int}) as a cut-off value to calculate the sensitivity and specificity of R_{int} to predict a 20% fall in FEV_1.

Results

At the first COMBO-study visit 73 children with moderate asthma (60% boys) with a median age of 9.2 years (range: 6.3 – 13.4 years) performed both R_{int} and FEV_1 measurements during methacholine challenge. Baseline characteristics are shown in Table 1. Fifty-two children reached the PD_{20} endpoint and were defined as responders (Table 2).

There was a significant increase from baseline of R_{int} starting at the sixth methacholine dose step and all dose steps thereafter (all $p<0.01$). From the first dose step mean FEV_1 was significantly different from baseline value (all $p< 0.01$), except for dose

Table 1. Baseline characteristics of children with moderate asthma (n=73) who performed a methacholine provocation test with both R_{int} and FEV_1 measurement at first visit.

		Median (interquartile range)
Age (years)		9.2 (7.6 – 10.6)
Height (cm)		135.6 (128.5 – 146.0)
Sex (number (%) males)		43 (58.9%)
FEV_1 [a]	z-score	-0.26 (-0.74 – 0.32)
	% predicted	96.8 (90.8 – 104.2)
FVC [b]	z-score	0.10 (-0.37 – 0.67)
	% predicted	101.4 (95.7 – 108.3)
FEV_1/FVC	z-score	-0.81 (-1.34 – -0.21)
	% predicted	94.1 (89.7 – 98.6)
R_{int} [c]	z-score	0.78 (0.05 – 1.57)
	% predicted	123.4 (107.0 – 147.2)

a) FEV_1 = Forced Expiratory Volume in 1 second; b) FVC = Forced Vital Capacity; c) R_{int} = Interrupter resistance.

Table 2. Number of children with moderate asthma (n = 73) who reached the PD_{20} endpoint [a)] at subsequent methacholine dose steps.

Dose step	Number
1 = 0.625 µg	0
2 = 1.25 µg	0
3 = 2.5 µg	0
4 = 5.0 µg	2
5 = 10.0 µg	6
6 = 20 µg	10
7 = 40 µg	10
8 = 80 µg	10
9 = 160 µg	9
10 = 320 µg	5
No threshold	21

a) PD_{20} endpoint = dose step at which a fall of more than 20% in FEV_1 was reached.

step 2. Visual inspection of individual R_{int} profiles during the challenge test showed large fluctuations with rises and falls at subsequent dose steps. In contrast, much smoother profiles with gradual falls were found for FEV_1 (see E-figure 1 in the online supplement). To express the variability in individual curves, the CV of measured values was calculated per patient. For R_{int} the mean CV of all patients was 10.6% (10^{th} - 90^{th} percentile: 5.5% – 16.3%). The mean CV for FEV_1 was significantly smaller: 2.9% (1.7% - 4.3%, $p<0.001$). Further analysis of CV R_{int} showed that there was no correlation with age (r_S = -0.13, p = 0.26). Also centres did not significantly differ from each other (Kruskal-Wallis test: p=0.17).

Analysing the 52 children who reached the PD_{20} endpoint, there was no significant correlation between changes from baseline of FEV_1 and changes from baseline of R_{int} at the last dose step at which the PD_{20} endpoint was reached (r_S =0.11, p = 0.44). Figure 1 demonstrates the mean changes from baseline of FEV_1 and R_{int} at each dose step, while at each dose step patients are grouped according to whether or not the PD_{20} endpoint was reached at the dose step. At none of the dose steps there was a significant difference in the mean change of R_{int} from baseline between those who reached the PD_{20} endpoint at that dose step versus those who did not, except at dose step 6 (p=0.014) (figure 1B). A combined analysis of all dose steps comparing responders and non-responders, using Cox regression analysis, showed that a greater change of R_{int} was associated with a higher probability of reaching the PD_{20} endpoint (p= 0.038). However, when inspecting the individual changes from baseline, there is large overlap of children who did and who did not reach the PD_{20} endpoint at the various dose steps (Figure 2). A ROC curve of changes from baseline of R_{int} as a measure to detect a 20% decrease in FEV_1 is shown in Figure 3, resulting in a small AUC of 0.65.

Figure 1. Mean changes from baseline of FEV_1 (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) was reached at that dose step. Error bars represent SEM.

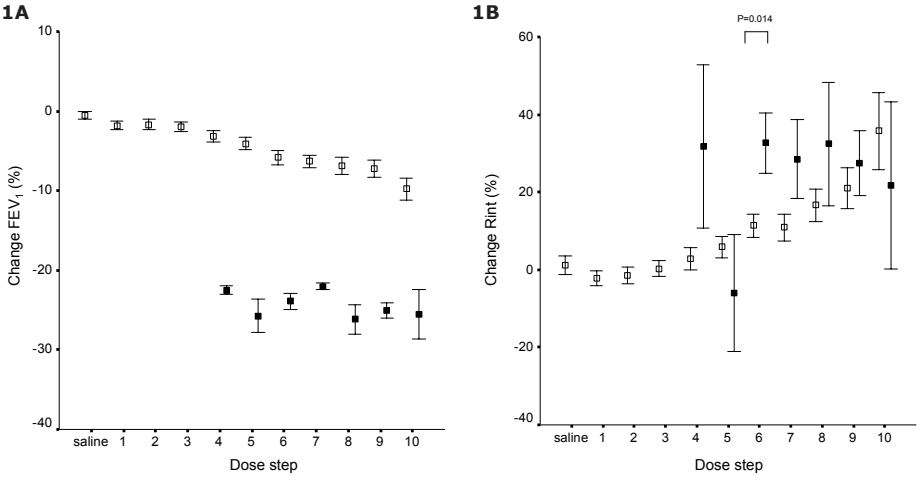


Figure 2. Individual changes from baseline of R_{int} at each dose step, divided according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) was reached. At each dose step there is large overlap in changes from baseline of R_{int} between both groups.

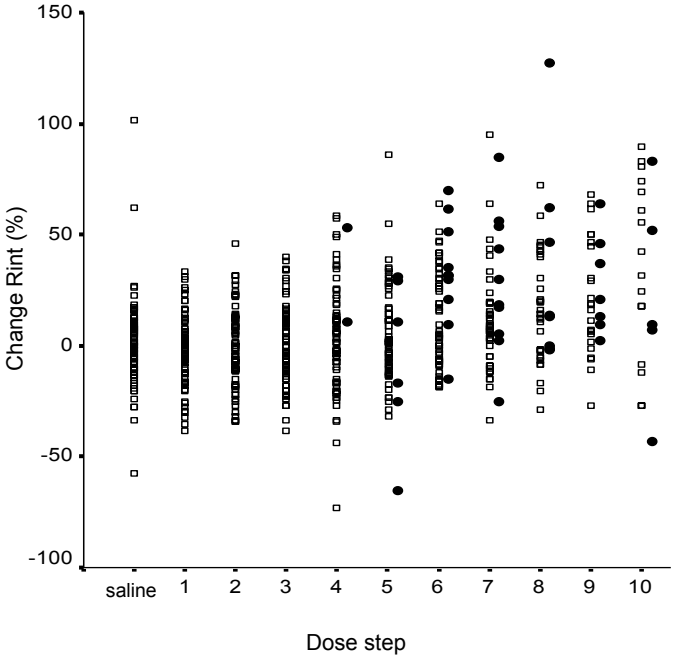
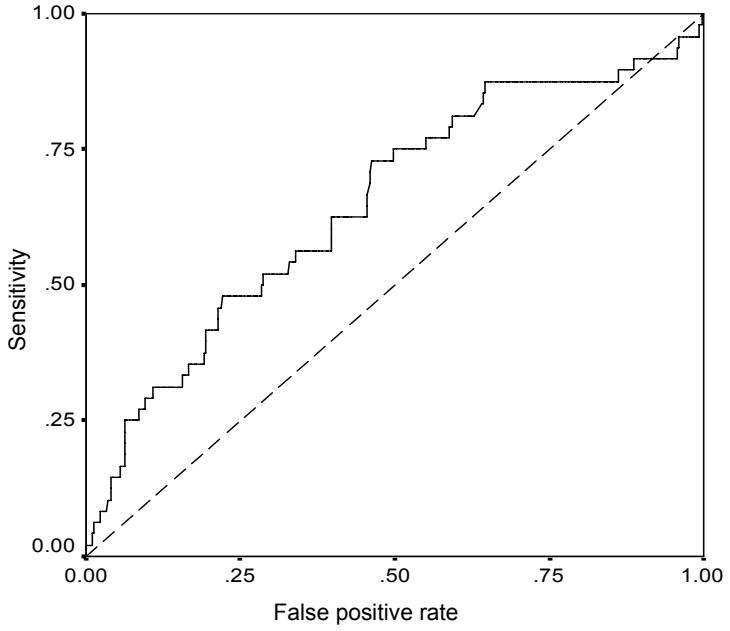


Figure 3. Receiver operator characteristic (ROC) curve showing the sensitivity and false positive rate of the change from baseline of R_{int} as a measure to detect a 20% fall in FEV_1 (PD_{20}). Area under the curve (AUC) = 0.65.



When using a 32.1% increase in R_{int} as cut-off value to calculate the sensitivity and specificity of R_{int} to predict a 20% fall in FEV_1, we found a sensitivity of 50% and a specificity of 43%. This means that R_{int} was able to detect half of the responders as determined by PD20 and that in 12 out of 21 children R_{int} increased with more than 32% without a concomitant fall of FEV_1 with 20% .

Repeating all analyses with change in R_{int} expressed as change in percentage predicted and change in z-score led to similar results (data not shown). AUC's of ROC curves for change in percentage predicted and for change in z-score were both 0.66.

In 64 children the methacholine challenge test was repeated after 26 weeks. The analyses to compare R_{int} with FEV_1 (as described above) showed similar results (data not shown). The AUC of the ROC curve was 0.61.

Discussion

This study showed that, although there was a significant increase of mean R_{int} values with higher doses of methacholine, individual changes in R_{int} are highly variable during methacholine challenge. The sensitivity and specificity of R_{int} to detect methacholine induced bronchoconstriction is low, which might limit the use of R_{int} in individual patients.

Several studies evaluated lung function measures requiring less patient effort than FEV_1, which could possibly be used as alternative measures of changes in bronchoconstriction. Klug et al showed that R_{int} was significantly less sensitive than specific airway resistance in detecting changes in airway function [7]. Phagoo et al found that R_{int} was also less sensitive and showed more variability than transcutaneous partial pressure of oxygen measurements [9;10]. In a study of Beydon et al. the majority of children with a 20% fall in transcutaneous partial pressure of oxygen ($PD_{20}P_{tc}O_2$) showed a significant change of R_{int} during methacholine challenge. However, all children who did not reach the $PD_{20}P_{tc}O_2$ had at least once a significant change from baseline [8], resulting in many false positive as well as false negative outcomes. Despite these data R_{int} is still advocated as an alternative measure for airway obstruction in those who are not able to perform forced breathing manoeuvres. Several studies showed significant correlation between gold standard FEV_1 and R_{int} in groups of patients [4;6], and reference values for R_{int} measurement in healthy controls have been published [15;17-20]. Although there is convincing evidence on the value of R_{int} measurement as a measure of airway obstruction in groups of patients, the sensitivity of R_{int} to detect changes in airway obstruction in individual patients has been little researched. Only one previous study compared R_{int} with FEV_1 during methacholine challenge testing [11] and found that on average R_{int} increased during methacholine challenge comparable to changes in FEV_1 measurements, but individual changes in R_{int} were not shown. Kannisto et al. compared R_{int} with FEV_1 during exercise challenge to diagnose BHR [21]. However, in this study only 11 (22%) children reached the R_{int} endpoint (15% increase from baseline) and only 6 of them also reached the FEV_1 endpoint (10% decrease from baseline). The children who showed a 15% rise in R_{int} but who did not have a 10% decrease in FEV_1 could be misclassified by R_{int} as having BHR. Davies et al. investigated the correlation between R_{int} and FEV_1 in bronchodilator response, and despite a correlation between FEV_1 and R_{int} in a group of patients, they found no significant correlation in individual children [22].

To evaluate the sensitivity and specificity of the R_{int} technique to detect changes in airway obstruction in individual children we performed simultaneous measurements of R_{int} and gold standard FEV_1 in a large group of children with asthma. We show that in individual cases the variability of R_{int} measurements is high (mean intra-patient CV 10.6%), precluding proper prediction of changes in FEV_1. In the present study the ROC curve, describing the sensitivity and specificity of R_{int} in detecting BHR as determined by the primary outcome FEV_1, had a small AUC of 0.65. This predictive capacity is lower compared to impulse oscillometry (AUC 0.68 to 0.75 [23]).

Large individual fluctuations in R_{int} might be due to large intrapatient short-term variation in airway resistance during tidal breathing [11;17;20;24]. This variability, resulting in low sensitivity and specificity, could limit the ability to detect thresholds in individual

airway obstruction as required in clinical practice. Furthermore the sensitivity might be lower compared to FEV_1 because during (severe) airflow obstruction equilibration of alveolar pressure might take longer than is realised during the shutter closure time during R_{int} measurement [25]. This may explain the lower R_{int} values in case of (severe) peripheral airway obstruction and hence lead to misclassifications during methacholine challenge testing. A third explanation of high variability of R_{int} might be the variability of upper airway resistance. Proper positioning of the head and neck and adequate support of the cheeks is highly warranted and might lead to intra-individual variability. The findings of our study imply that on group level BHR can be documented, since we showed on average a significant rise in R_{int} with increasing methacholine doses. This may be useful in epidemiologic research. However, R_{int} seems to have insufficient capacity to diagnose BHR in individual patients and therefore it is of limited value in BHR measurements in clinical practice.

A major strength of our study is the fact that the methacholine challenge test was repeated after 26 weeks and resulted in a similarly low sensitivity and specificity of R_{int}, which validated the results. Beside this, the present study may have some limitations. Firstly, the study was performed in children with a mean age of 9.3 years, because preschool children are seldom able to perform FEV_1 measurements. It could be questioned if the found results are applicable to younger children. Since R_{int} is a measure of total airway resistance, an induced bronchoconstriction will lead to a relative larger increase in R_{int} in young children compared to older children, because in young children lower airways resistance contributes more to total airway resistance [26]. Nevertheless, the fluctuations in R_{int} are expected to be similar as the short term repeatability of R_{int} that has been found to be independent of age[20;27]. Additionally we used a 20% fall as 'gold standard' in detecting methacholine induced bronchoconstriction. When we used a 32% increase in R_{int} (the coefficient of repeatability of R_{int} measurements as described by Beydon et al.) as a cut-off value to determine BHR, there were 12 children with a significant change in R_{int} but without a 20% fall in FEV_1. The pathophysiology of a fall in FEV_1 during a forced manoeuvre might be different from an increase of R_{int} during tidal breathing. Therefore, there is a possibility that FEV_1 might not be the gold standard for bronchial challenges especially in young children.

The current study showed on average an increase of R_{int} with subsequent methacholine doses during methacholine challenge tests and therefore it might be useful as a measure of airflow obstruction in groups of patients, e.g. in large epidemiological studies. However, we conclude that R_{int} seems to have insufficient capacity to diagnose BHR in individual patients.

Acknowledgements

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Online supplement

Methods

This study was a substudy performed in some centres participating in the multicentre COMBO-study. The COMBO-study is a blinded study comparing the clinical effects of fluticasone propionate 200 mcg BD versus salmeterol/fluticasone propionate 50/100 mcg BD on number of symptoms free days in children between 6 and 16 years of age (trial registration number NCT00197106; GSK study number SAM101667) (1). It was performed in 158 patients in 19 hospitals in the Netherlands, between June 2005 and October 2008. All children had a clinical history of moderate asthma (according to NAEPP guidelines (2)) and bronchial hyperresponsiveness (BHR) (methacholine dose at which at least 20% fall in FEV_1 (PD_{20}) ≤ 150 µg). Nine hospitals performed additional Rint measurements during methacholine challenge tests, so children from these centres were included in this comparative lung function study. The study was approved by the local medical ethical committees of the different centres and written informed consent was obtained from the parents (and also from the child if ≥12 years old) before the start of the study.

Measurements

Methacholine challenge testing

Methacholine challenge tests were performed using the dosimeter method (3;4) with a nebulizer (model 646, DeVilbiss Healthcare, Somerset, USA) attached to a dosimeter (Rosenthal French dosimeter, PDS Instrumentation, Louisville CO, USA). After baseline R_{int} and FEV_1 measurements, 0.9% NaCl was inhaled to rule out non-specific reactions and additionally methacholine chloride was administered in doubling doses (0.625 µg, 1.25 µg, 2.5 µg, 5.0 µg, 10.0 µg, 20.0 µg, 40.0 µg, 80 µg, 160 µg, 320 µg). The procedure was performed according to the latest ATS/ERS guidelines (4) as follows: after manually triggering of the dosimeter children were instructed to inhale slowly from forced residual capacity (FRC) to total lung capacity (TLC) and then hold their breath for 2 seconds. Two minutes after each inhalation, R_{int} and FEV_1 were measured. Provocation was continued after at least five minutes and until the PD_{20} or the maximum dose was reached.

Interrupter resistance

Airway resistance measurements were carried out using the MicroRint (Micro Medical Limited, Kent, UK) that contains a pneumotachometer, a flow interruption valve

and a pressure transducer to measure mouth pressure post-occlusion. A bacterial filter was used (Spirosafe bacterial filter, Micro Medical, Kent, UK). Measurements were performed conform the latest ATS/ERS guideline (5). The child was seated and diverted to ensure quiet tidal breathing. Children were breathing through a mouth piece while the technicians supported the cheeks to reduce the change in upper airway compliance. Ten interruptions of 100 ms were made at peak expiratory flow with a random frequency, with the valve closing within 10 ms. The MicroRint software automatically rejected measurements that showed an artefact on the pressure curve. Additionally measurements that showed tachypnoea or irregular breathing, vocalisation, or hyperextension or flexion of the neck were manually discarded as well as were tracings with a horizontal or declining pressure signal suggesting air leakage around the mouthpiece. R_{int} values were calculated using the two-point linear fit back extrapolation technique to t=15ms (6). A minimum of five successful interruptions was required to calculate the median R_{int} value. Reference values of Merkus et al. (7) were used to convert R_{int} outcomes into percentage predicted values.

Spirometry

Maximal expiratory flow-volume measurements were performed with standardized equipment. Seven centres used a Jaeger pneumotachometer (Viasys Healthcare, Hochberg, Germany), 1 centre used the Microloop (Micro Medical, Kent, UK) and 1 centre used a Zan spirometer (nSpire Health GmbH, Oberthulba, Germany). Measurements were performed according to the latest ATS/ERS guidelines (8) with the child sitting and wearing a nose clip, without using a bacterial filter. Equipment calibration was performed daily and all measurements were BTPS corrected. After a full inspiration children performed a maximally forced and full expiration. At least three technician-accepted flow-volume curves were obtained and the largest FEV_1 was selected. Reference values of Stanojevic (9) were used.

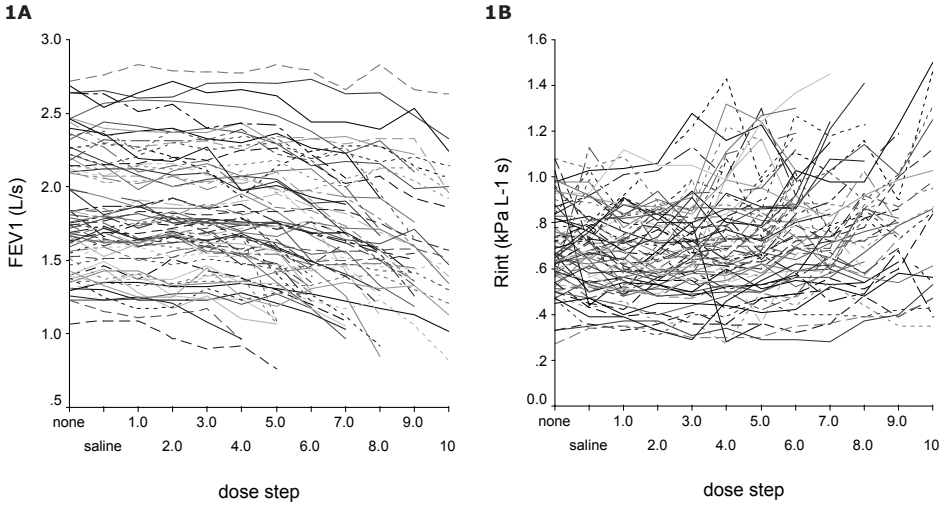
Analysis; Calculation of intrapatient coefficients of variation (CV):

To quantify the variability of FEV_1 and R_{int} measurements between the various dose steps during the methacholine provocation test, a coefficient of variation (CV) was calculated for each separate patient. This was done by fitting smooth third degree polynomials of each parameter separately versus log-methacholine dose for each individual patient to allow a gradual change over the subsequent dose steps. The resulting standard deviation of residuals for each patient was subsequently divided by the same patients mean value to obtain an individual CV of the measurements. The resulting individual paired CV's of FEV_1 and R_{int} were compared using Wilcoxon's test.

Results

Visual inspection of individual R_{int} profiles during the challenge test showed large fluctuations with rises and falls at subsequent dose steps. In contrast, much smoother profiles with gradual falls were found for FEV_1 (E-figure 1).

Figure 1. Individual fluctuations of absolute values of FEV_1 (1A) and R_{int} (1B) plotted against dose steps of methacholine in 73 children with moderate asthma. Dose step 0.5 denotes Saline.



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CHAPTER 8

Comparing 6 and 10 seconds exhalation time in exhaled Nitric Oxide measurements in children

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Abstract

Introduction

Standard exhalation time for Fractional exhaled Nitric Oxide (FeNO) measurements is 10 seconds but this is difficult for young children. Recommended exhalation time for children is 6 seconds, but this was never substantiated in literature. We aimed to investigate the agreement between FeNO values measured with exhalation times of 6 and 10 seconds and the preference of children for either method.

Methods

The study population comprised children aged 5-17 yrs visiting the Pediatric Pulmonology outpatient clinic. FeNO values, measured during 6 (FeNO-6) and 10 (FeNO-10) seconds (random order) using the single- breath online (SBOL) technique, were compared. Preferences for exhalation times were related to FVC values.

Results

Ninety-eight children (mean age 10.6 yrs) were included. Median FeNO-6 (15.2 ppb) and FeNO-10 (13.6 ppb) did not differ significantly (p= 0.259). Mean difference between FeNO-6 and FeNO-10 was –0.3 ppb, limits of agreement ranging from –5.8 ppb to +5.3 ppb. 60% of children with a Forced Vital Capacity (FVC) less than 3 liters preferred the FeNO-6 method.

Conclusion

We found good agreement between FeNO-6 and FeNO-10, so they can be used interchangeably. An exhalation time of 6 seconds was preferred by the majority of subjects with a FVC below 3 liters.

Introduction

Nitric Oxide (NO) is an inflammatory marker in the airways, first described in 1991 by Gustafsson et al [1;2]. Patients with asthma show higher NO concentrations in exhaled air [1] and fractional exhaled Nitric Oxide (FeNO) is currently used as a routine marker to diagnose and monitor asthma.
FeNO is preferably measured using the single-breath online (SBOL) technique. However young children may have difficulty exhaling long enough for adequate NO wash out from the lower airway compartment and to achieve a stable FeNO plateau resembling actual airway NO production [3]. Most commonly used FeNO measuring systems require an exhalation of 10 seconds, and FeNO is expressed as the mean NO concentration during the last 3 seconds. According to ATS/ERS recommendations a 6 seconds exhalation would suffice in children of all ages [1;3], but this was never substantiated in literature. Most studies use exhalation times of 10 seconds, not only in children above age 12 [4;5], but sometimes below age 12 as well [5;6], which might have reduced success-rates.
We set out to investigate the agreement between FeNO measures with exhalation times of 6 and 10 seconds and the preference of children aged 5-17 years for either method.

Methods

A cross-sectional study was performed at the outpatient clinic of the Pediatric Pulmonology department of the Wilhelmina Children's Hospital in Utrecht, the Netherlands. Children aged 5-17 years were included between September 2007 and February 2008. All children visited the outpatient clinic because of (the suspicion of) pulmonary diseases and a FeNO measurement was requested by their pediatrician as part of routine clinical care. We obtained oral informed consent from all the children and/or their parents.

Measurements

FeNO was measured using the SBOL method for children according to the latest ATS/ERS statements [1;3], using the NIOX system (Nitric Oxide Monitoring System; Aerocrine, Sweden). The inspired air passed a NO-scrubber to ensure that NO-free air was inhaled.
Children inhaled through the mouthpiece to near total lung capacity (TLC) and subsequently exhaled with a constant flow of 50 ml/s against a pressure between 10 and 20 cm H_2O to prevent contamination of the sample with air from the upper

airways. All measurements were performed in a comfortable sitting position without wearing a nose clip. A visual feedback was used to ensure proper flow during exhalation. Conform ATS/ERS statements repeated measurements were performed (2 values that agree within 5% or 3 that agree within 10%) with at least 30 seconds intervals. Mean FeNO was recorded in parts per billion (ppb).
All children performed the above described procedure during both 6 (FeNO-6) and 10 (FeNO-10) seconds exhalation. FeNO was measured during the last 2 seconds (FeNO-6) and 3 seconds (FeNO-10) of the maneuver, respectively. To avoid a possible learning effect, children were randomized to start with either of the two methods. Children who did not successfully perform the first measurement were excluded. Following FeNO measurements the children were asked which method they found easier to perform.
Spirometry was performed using a pneumotachometer system with a heated Lilly head (Viasys Healthcare, MasterScreen, Hochberg, Germany). Measurements were carried out with the child sitting straight and wearing a nose clip. Three technically acceptable curves were obtained and Forced Vital Capacity (FVC) was derived from the best maneuver.

Data analysis

Statistical analysis was performed using SPPS-12.0 (SPSS, Chicago, Il). Subjects who were not able to perform the second measurement were excluded from further analysis. Differences between FeNO-6 and FeNO-10 were skewed (Kolmogorov-Smirnov test of normality; $p< 0,01$), and therefore FeNO-6 and FeNO-10 were compared using the Wilcoxon-rank-sum test and correlated using Spearman rank correlation. The agreement between FeNO-6 and FeNO-10 was assessed with Bland and Altman analysis [7]. Relations between the order of the two measurements and the preferences for different methods were analyzed using a Chi-square test. Children's preferences for exhalation times were recorded in percentages for different ages and for different FVC values.

Results

Ninety-eight children (54 boys, 44 girls, mean age 10.6 years (sd 3.1 yrs)) successfully performed the first measurement, ninety-three performed both. Those children who could not perform the 10 seconds but did perform the 6 seconds method, were all below age 12. The numbers of participants at different ages are shown in table 1.

Table 1. Number of children participating at different ages

Age (yrs)	Number
5-6	9
7-8	16
9-10	23
11-12	14
13-14	17
15-17	14

Table 2. Median and interquartile range (IQR) of FeNO-6 and FeNO-10 values (ppb) of total group and for different diseases.

		Median	IQR
Total group (n=93)	**FeNO-6** [a]	15.2	9.6 – 29.5
	FeNO-10 [b]	13.6	9.8 – 27.6
Asthma or atopy (n=67)	**FeNO-6**	18.7	11.2 – 38.0
	FeNO-10	18.5	10.4 – 37.0
Cystic Fibrosis (n=12)	**FeNO-6**	10.6	8.9 – 11.4
	FeNO-10	9.9	8.0 – 11.6
Other [c] ***(n=14)***	**FeNO-6**	11.6	7.1 – 17.2
	FeNO-10	10.2	6.4 – 17.1

[a]FeNO-6: Fractional exhaled Nitric Oxide during 6 sec exhalation time
[b]FeNO-10: Fractional exhaled Nitric Oxide during 10 sec exhalation time
[c]Other: Recurrent infections of lower and upper respiratory tract (n=10), anatomical abnormalities (n=2) and functional breathing disorders (n=2).

Sixty-seven of the 93 children were diagnosed with asthma or atopy, 12 children with cystic fibrosis and 14 were classified as "other". This category contains children diagnosed with recurrent infections of lower and upper respiratory tract (10), anatomical abnormalities (2) and functional breathing disorders (2). Table 2 presents FeNO values in children with different diseases. Median FeNO-6 (15.2 ppb) and FeNO-10 (13.6 ppb) did not differ significantly ($p= 0.259$).

Both methods were significantly correlated ($r = 0.976$, $p < 0.01$). Mean difference (FeNO-10 minus FeNO-6) was –0.3 ppb, with "limits of agreement" ranging from –5.8 ppb to +5.3 ppb (Figure 1).

Children's preference for either method was unrelated to measurement order. Figure 2 shows preferences distributions for the different exhalation times at different FVC values. With increasing FVC the preference for 6 seconds decreased while it increased for 10 seconds. In children with an FVC below 3 liters (median FVC at 12 yrs = 2,7 liters, median FVC at 13 yrs = 3,4 liters), 60% preferred a 6 seconds exhalation versus 31% of the children with an FVC above 3 liters. 26% of children with FVC< 3 liters preferred the 10 seconds exhalation, versus 50% of children with FVC> 3 liters.

Figure 1. Bland-Altman plot of Fractional exhaled Nitric Oxide measured during 10 sec (FeNO-10) and Fractional exhaled Nitric Oxide measured during 6 sec (FeNO-6). Difference between FeNO-6 and FeNO-10 is expressed as FeNO-10 minus FeNO-6.

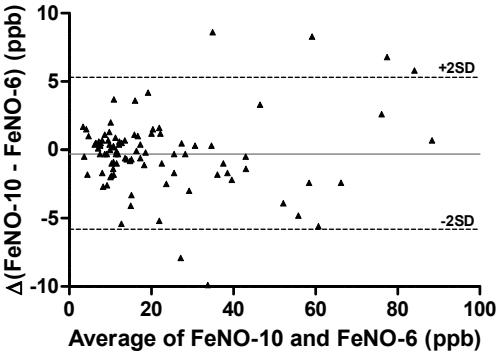
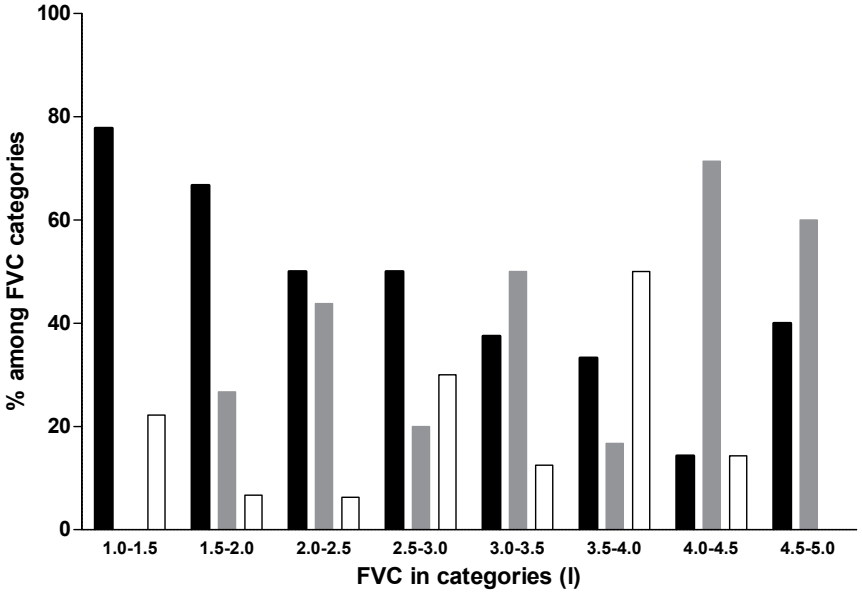


Figure 2. Preference for exhalation time according to FVC categories. Preference for 6 sec (black), 10 sec (grey) and no preference (white). FVC= Forced Vital Capacity. Increasing preference for a 6 sec exhalation time with decreasing FVC.



In the group of children below the age of 12 years, 68% preferred the 6 seconds method, versus 25% in older subjects. 21% of younger children preferred the 10 seconds method versus 53% in older subjects.

Discussion

This study investigated the agreement between the results of FeNO measurements during 6 and 10 seconds exhalation. FeNO-6 and FeNO-10 showed excellent agreement, which indicates that both methods can be used interchangeably. Children with vital capacities below 3 liters prefer a shorter exhalation time.

Our results agree with other recent findings [8]. For several reasons we consider the differences between FeNO-6 and FeNO-10 irrelevant for clinical practice. Kharitonov et al. demonstrated that FeNO measurements are highly reproducible [9]. The differences we measured between FeNO-6 and FeNO-10 (–5.8 ppb to +5.3 ppb) were similar to differences they found between repeated measurements (–4.0 ppb to +4.0 ppb). This means that the variability in FeNO found in the two different methods is comparable to the variability of FeNO within subjects.

Secondly there is a large variability of FeNO between subjects diagnosed with asthma. Reported FeNO levels in asthmatic children range from mean 22 ppb [10] till 78 ppb [11]. A difference of 5 ppb between FeNO-6 and FeNO-10 is therefore small in relation to the broad reference range for asthmatics.

Thirdly, there is overlap in FeNO values between non-asthmatic and asthmatic children[9]. Patients with mild asthma, especially non-atopic asthma, can have 'normal' FeNO[12], whereas non-asthmatic children with an allergic rhinitis may have an increased FeNO[13]. Taylor et al. developed an algorithm for FeNO as an aid in diagnosing chronic respiratory symptoms [12]. In children they consider a diagnosis of asthma very likely when FeNO is higher than 35 ppb, suggest another diagnosis at values lower than 20 ppb and to base diagnosis on clinical presentation when FeNO is between 20 and 35 ppb. For differentiating healthy from asthmatic subjects, the difference we found between FeNO-6 and FeNO-10 will not influence diagnosis.

There is no literature on children's preference for different exhalation times. We found those with low vital capacities to prefer the 6 seconds method more often. A low FVC can result in more difficulties in exhaling at a constant flow of 50 ml/s during 10 seconds. For that reason we expected no preference of children with a high FVC. However, several children reported to prefer an exhalation of 10 seconds because the visual-feedback-incentive moved more slowly and more stable when adjusting for the right flow.

Contrary to ATS/ERS statements we did not use 4 seconds exhalation in children under age 12. We choose an exhalation time of 6 seconds because this is the standard setting in many currently available NO measurement devices (like the NIOX-mino).

We conclude that measurement of FeNO during 6 seconds is comparable with the 10 seconds method in children aged 5-17 yrs. Particularly, in children with vital capacities below 3 liters, measurement of FeNO during 6 seconds is preferred.

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CHAPTER 9

General discussion

Main findings

The aim of this thesis was to investigate the influence of genetic and environmental factors on lung function in healthy children at different ages and to provide new reference values for the most commonly used lung function tests throughout childhood and adolescence. Additionally, we aimed to evaluate the use of two specific lung function tests for young children.
In the first part of this thesis determinants of early life lung function were considered. We investigated the influence of prenatal smoke exposure and parental lung function on neonatal lung function in a cohort study of healthy newborns. During follow-up of this cohort, we evaluated whether the influence of prenatal smoke exposure was still present at the age of five. Furthermore, we assessed the impact of postnatal smoke exposure and rapid early life weight gain on childhood lung function. In the second part of this thesis, we provided new reference values for the most commonly used lung function tests in children. Finally, we determined whether the interrupter resistance measurement could be used in bronchial provocation testing and we evaluated the feasibility of exhaled Nitric Oxide measurements in young children.

In summary, the main findings of this thesis are:

- In healthy newborns neonatal lung function is associated with parental lung function, even after correction for familial aggregation of body size and shared environment.
- Children with accelerated weight gain during the first 3 months of life have more wheezing complaints in early childhood and decreased lung function at the age of five.
- The implementation of smoke free legislation results in less pregnant women being exposed to environmental tobacco smoke, and a concomitant rise in birth weight and birth-weight-adjusted neonatal lung function.
- Prenatal tobacco smoke exposure results in reduced neonatal lung function in healthy newborns, but this does not persist into childhood when there is no postnatal smoke exposure.
- Parental smoking during childhood results in diminished airway function in healthy children, with the most marked decrease in peripheral airway function.
- New reference values for paediatric lung function tests provide a more valid estimation of a patient’s lung function and less over- and underdiagnosis of pulmonary diseases.
- Measurement of interrupter resistance is no alternative for forced expiratory volume measurements to diagnose bronchial hyperresponsiveness in individual patients.

- An exhalation time of six seconds during exhaled Nitric Oxide measurements showed good agreement with the standard exhalation time of 10 seconds and is favoured by young children.

In this chapter we further discuss some general aspects and implications of our findings.

The program of lung growth; tracking and detracking of lung function in early life

In the previous years, several large cohort studies found associations between lung function in childhood and adulthood[1-4]. This suggests that lung function 'tracks' throughout life, meaning that on average an individual's position in the lung function distribution, relative to peers, remains stable over time. Since prenatal and early postnatal life are the most critical periods in terms of lung growth and development, exposures in these periods of life could result in 'detracking' from the innate lung function level, meaning that someone's lung function position relative to peers could change.
The theory of 'programming'[5;6] of lung structure and function suggest that once detracking has occurred in prenatal or early postnatal life, this will have lasting consequence, meaning that someone's lung function position will subsequently track along a higher or lower percentile line than inherited. In other words, the lung function level reached at the end of the first years of life would determine the course of lung function development, as well as the final lung function at the end of adolescence. On the contrary, there are studies showing that a certain degree of "catch-up" growth of lung function is possible. In experimental settings it was shown that the rate of lung growth can be accelerated in the postnatal period[7]. Furthermore, there is increasing clinical support of "catch-up" lung growth in children when their lung disease is no longer active[7]. This would imply that the effect of early extrinsic exposures does not per definition need to be permanent.

We showed that neonatal lung function can only be partially explained by familial aggregation of body size and shared environmental factors. This means that to some extent, genes determine neonatal lung function and the starting level of lung function. Since growth is one of the major determinants of lung function in both prenatal and postnatal life, a genetically stipulated growth pattern plays an important role in tracking of lung function.

When no extrinsic exposures would be present, the congenital lung function and growth pattern would determine someone's position in the lung function distribution and the stability of lung function over time.
However, apart from intrinsic factors determining stability of lung function, there are extrinsic exposures that can alter the course of lung growth and development. Obviously, the factors that can induce downward change of lung function, such as lung disease or adverse environmental exposures, or upward change of lung function, possibly reflecting lung function recovery from previous insults, are of great scientific interest.
By generating new reference equations for lung function measures, we showed that, due to secular trends in body growth, average lung function increased over the past decades. On the other hand, parallel to studies in the cardiovascular area, we showed that accelerated weight gain in the first three months of life was associated with decreased lung function at the age of five. One could question whether accelerated weight gain during early life is caused by unnecessary overfeeding, resulting in overweight or adipositas or whether this catch-up growth reflects the need to shift to someone's genetically determined level of body composition after a prenatal period in which insufficient nutrients were available to reach this level. The first explanation implies a direct effect of weight gain and could be supported by studies showing a relationship between adipositas and reduced lung function in adults and children[8-11]. However, since we did not collect data on the course of weight gain during the first five years of life, we cannot tell whether this early life weight gain is the cause of a permanent impaired lung function ('programming'), or whether there was ongoing acquisition of adipose tissue. The second explanation implies that excessive postnatal growth reflects adverse conditions in utero, leading to a lower birth weight than what would have been genetically expected. As a result, impaired growth in utero results in catch-up growth after birth, possibly shifting to someone's genetically determined level of body growth. In this scenario, excessive body growth itself does not cause reductions in lung function, but the adverse prenatal conditions result in impaired lung development in utero, apparently without postnatal catch-up growth of lung function. This could support the 'programming' theory that adverse events in early life have lasting effects. However, since we did not longitudinally study the effect of rapid weight gain on lung function in this cohort, we could not prove that the effect is permanent. We do not know if lung function at earlier age was equally reduced, nor if this reduction will persist into further childhood. Further longitudinal research should address the question whether the negative effect of accelerated weight gain in early life has waning effects on lung function or whether this effect persists throughout childhood.
By longitudinally studying the influence of smoke exposure on lung function we investigated whether early life exposure to tobacco smoke has a permanent influence

on lung function level, supporting the 'programming' theory, or whether the lungs have redeveloping capacities once the exposure disappears, resulting in no more than a temporarily reduction of lung function. We showed that lung function of children who were exclusively prenatally but not postnatally exposed to tobacco smoke, was reduced at birth but the effect of prenatal smoke exposure waned during the first five years of life. However, when smoke exposure persisted after birth, less catch-up lung growth was found and consequently lung function remained impaired. Since prenatal and postnatal environmental tobacco smoke exposure are strongly correlated, most prenatally exposed children will be continuously exposed to environmental tobacco smoke during childhood, resulting in a reduction in lung function that is present throughout childhood.

Implications for clinical practice

If programming of lung growth can be influenced by non-genetic factors, this might create opportunities to influence pulmonary health in later life. In this thesis the main factors under investigation were early growth and smoke exposure.

We have confirmed previous findings that rapid growth in early life is associated with more respiratory symptoms and decreased lung function in childhood. However, before these findings can result in a recommendation for clinical practice, they first need to be confirmed in a clinical trial. Children in whom feeding and growth is regulated by adapting the amount of formula feeding based on their individual growth curve could be compared with children in whom feeding is not strictly regulated. When regulated growth to prevent excessive growth in the first months of life results in less respiratory symptoms and a better lung function, this could imply that growth should be more strictly supervised, by giving more individualized advices, although this is definitely more difficult when children are breastfed than when children get formula feeding.

The found relationship between the incremental implementation of smoke-free legislation and increased neonatal lung function, demonstrated that respiratory health can be improved by preventive measures in early life. Although we showed that the negative effect of prenatal smoke exposure on lung function waned during the first years of life when children were not postnatally exposed to tobacco smoke, this is a very theoretical situation, because most women who are unable to quit smoking during pregnancy will continue smoking afterwards. Therefore, pregnant women should be encouraged to avoid active as well as passive smoking. Additionally, parents should be recommended to create a smoke-free home. Even when children are prenatally exposed to tobacco smoke, the adverse effect on lung function can disappear when postnatal smoke exposure is avoided, so creating a smoke-free home, even after birth, seems favourable. A possible adventitious consequence of a smoke-free environment is that fewer children will copy smoking habits from their

parents. This will lead to less smoking in adolescence as well as in adult life, resulting in a higher peak level of lung function at the end of adolescence and less lung function decline during adulthood.
Besides the positive effects of a smoke-free environment on the lung function of children, prevention of smoke exposure will probably result in more health benefits. In previous studies, environmental tobacco smoke was shown to be associated with more respiratory complaints in children[12;13]. Furthermore, prenatal smoke exposure was correlated with higher systolic blood pressure at birth[14], obesity in childhood[15] and a higher carotid artery intima-media thickness in young adults[16]. The latter are possible risk factors that also show tracking over time. A better cardiovascular risk profile in childhood will therefore probably result in less adults suffering from cardiovascular diseases. The government should play a major role in further reducing smoke exposure during pregnancy by implementing and controlling smoke-free legislation as well as by smoke-free health campaigns. In this way more women and their offspring could benefit from the positive health effects of a smoke-free environment.

Implications for future research

The major drawback of studies regarding tracking of lung function is that longitudinal studies from prenatal period to old age are lacking. Most longitudinal studies on lung function tracking only cover a limited period and not the complete period of lung growth and development[1;2;4], except for the study by Stern et al in which lung function was measured longitudinally from infancy until the age of 22[3]. Most of the studies describe tracking of lung function within children with persistent wheeze[2] or severe asthma[1], showing that children within these subgroups have a constantly reduced lung function. They do not describe the normal pathophysiological course of tracking and especially detracking in healthy children. Therefore, those studies are unable to answer the question whether extrinsic exposures can program the course of lung development. More studies in healthy children are needed to investigate whether early life exposures result in detracking of lung function level, with subsequent tracking of that level throughout childhood, or whether some exposures cause only a temporarily detracking, with catch-up growth once the exposure disappears. Since there has been only one prior longitudinal study investigating the effect of prenatal smoke exposure on lung function changes in early childhood[17;18], future studies are needed to confirm the waning effect of prenatal smoke exposure on lung function.

This thesis studied tobacco smoke exposure and excessive body growth as possible determinants of detracking. The positive findings suggest that also other factors might play an important role. Positive and negative detracking could possibly be initiated by e.g. physical activities, medications and a calorie-rich diet. Future studies could

investigate whether physical activities at a young age will result in positive detracking of lung function and whether this effect would be permanent or temporarily once sporting is ceased. On the other hand one could question whether inactivity in early childhood could lead to negative detracking. In the same way, research could focus on, for example, the use of inhaled corticosteroids in wheezing children and whether the positive effect on lung growth and development would be persistent, even when medication is discontinued. Furthermore, the effect of rapid weight gain should be longitudinally studied to show if one period of rapid weight gain have lasting effects on respiratory health or whether these effects wane once growth along a percentile line has been established. When adipositas is related to reduced lung function, another question to be answered could be whether lung function improves with losing weight. Although parental lung function predicts the congenital level of lung function in offspring, studies investigating genetic polymorphisms associated with lung function will be helpful to understand the heritability of lung function. Although some genetic polymorphisms associated with lung growth are known (e.g. single nucleotide polymorphisms in the ADAM33 gene[19], most genetic factors that affect lung growth still need to be discovered. Furthermore, studies on gene-environment interactions are needed to improve the knowledge on genes that make individuals more susceptible for environmental factors that can permanently or temporarily change lung growth. The response to an extrinsic factor is at least partially determined by someone's genetic profile. There have already been identified several genetic polymorphisms that result in worse respiratory outcome when prenatally exposed to tobacco smoke[20-24]. Further research could possibly evaluate whether a certain genetic background could predispose for persistent reductions in lung function once exposed to tobacco smoke. Finally, the factors involved in detracking of lung function could also influence development of other organ systems. For example, it would be interesting to investigate whether smoke exposure has persisting or temporarily effects on the development of other organ systems, such as the cardiovascular system. In the WHISTLER study the effect of prenatal smoke exposure on blood pressure could be longitudinally studied to investigate whether the effect of prenatal smoke exposure on cardiovascular risk factors wanes or persist throughout childhood. In general, more longitudinal studies are required to investigate whether exposures in early life have permanent effects on organ growth and development.

Clinical intermezzo

Case 1

A boy with cystic fibrosis. When his lung function is measured throughout adolescence, the following values are found:

- *At the age of 14, his height is 1.70 m and his FEV_1 is 3.24 L.*
- *At the age of 17, his height is 1.74 m and his FEV_1 is 3.36 L.*

With the previously used reference values of Zapletal, one would conclude that his FEV_1 in percentage from predicted decreased only just 3% from 93% to 90%. With the new predicted values, his lung function shows a more pronounced deterioration from 87% to 79% from predicted (from the 12th percentile to the 3rd percentile) which would urge to more aggressive treatment. His lung function is now below the lower limit of normal using the new reference values, which is considered abnormal, whereas with the previous reference values, his FEV_1 value would still be judged as normal.

Case 2

A 5-year-old girl suffers from wheezing episodes. Her mother tells that the wheezing episodes do not respond very well to bronchodilator therapy, her general practitioner refers her to the paediatric pulmonology outpatient clinic for lung function testing. Her symptoms are evaluated and are suspect for asthma. There are no abnormal findings at physical examination.

- *A forced flow-volume measurement shows normal FEV_1 of 1.22 L, with a concave shape of the expiratory loop and a FEF_{25-75} of 1.23 L/s. This is 77% from predicted when using Zapletal and 79% from predicted and at the 20th percentile when using the new reference equation.*
- *The curve showed significant improvement (FEV_1 increased with 15%) after inhalation of a bronchodilator.*
- *Fractional exhaled Nitric Oxide measurement is initially tried with a 10 seconds measurement, which is unsuccessful. The mode is switched to a 6 seconds exhalation time, resulting in a mean FeNO of 46 ppb.*
- *At the following visit, a bronchial challenge test is performed, showing mild bronchial hyperresponsiveness with a decrease in FEV_1 of 23% after inhalation of 4.0 mg/ml methacholine.*

The diagnosis of asthma is confirmed and treatment is started. The mother asks why the lung function tests could not have been performed at the age of three when the wheezing episodes started. Furthermore, she had these complaints herself when she was a child but they disappeared when she became older. When she was a child, she used to have a peak flow meter at home but her lung function had never been tested in a lung function laboratory.

The use of lung function tests in paediatric clinical practice

Lung function tests can contribute in the diagnosis and follow-up of children with lung diseases, such as asthma and cystic fibrosis, and other diseases influencing lung function such as neuromuscular diseases and children who received a bone marrow transplantation. Furthermore, lung function tests are used to evaluate candidates for lung transplantation.

Reference values

Reference equations are used to calculate a patient's predicted lung function based on the lung function of healthy individuals of the same height, age and sex. The chosen reference values importantly influence the interpretation of lung function tests, resulting in misinterpretation of lung function outcome when outdated or non-representative reference equations are used.

Because of secular trends in health and nutrition, the new reference values generally turned out higher than previous reference values. This is shown in case 1, in which the FEV_1 value is still 90% from predicted at the age of 17 when using the previous reference equations by Zapletal[25]. When using the new reference values, the FEV_1 value is already below the lower limit of normal, and therefore considered abnormal. Furthermore, by including age and sometimes the interaction between height and age in the reference equations, we were able to more correctly model the nadir in lung function during puberty than studies omitting age as one of the predictors of lung function. Since age is a substitute for maturation, lung function still increases when length growth slows down at the end of the growth spurt but thorax size still increases, especially in boys. As shown in case 1, the use of the previous reference equations, which did not include age, resulted in a decrease of FEV_1 of only 3%, whereas the new reference equations showed a decrease of 8% over time.

Moreover, we succeeded in predicting more precisely the coefficient of variation of lung function and provide a valid estimate of the lower limit of normal, so that the use of a fixed value as lower limit of normal can be avoided. When the between subject variability is large, the lower limit of normal as expressed in percentage predicted could be much lower than the commonly used cut-off value of 80%. The difference between a measured and predicted lung function value, expressed as percentage predicted, does not inform the clinician on how unusual this value is. On the other hand, the new reference equations provided percentile scores that tell how infrequent a value occurs in the current population, by expressing the percentage of children with a lung function above or below a patient's lung function.

This is shown in case 2, in which the girl has an FEF_{25-75} value below the cut-off of 80% from predicted, but because of the large variation in this measure, the value is on the 20th percentile, and therefore within the range of normality.

Diagnosis and follow-up

The extent to which a lung function test is useful in diagnosing lung diseases in clinical practice depends on the discriminative capacity of the lung function test in an individual patient. Due to the large overlap between health and disease, the diagnostic accuracy of a lung function test is generally very poor. This is shown in case 2, in which the girl is diagnosed with asthma, but has an FEF_{25-75} value at the 20th percentile.

Furthermore, the usefulness of a lung function test in follow-up of lung diseases depends on the short and long term variability of the test. In order to distinguish whether a change in lung function can be ascribed to an intervention, an exacerbation of a disease, or to normal variation in the lung function measurement, one should know the intermeasurement variability of the lung function test, expressing the intrinsic variability of the measurement or disease. The coefficient of repeatability (CR) represents the intermeasurement repeatability, computed as twice the standard deviation of the mean of two measurements. When we used the previously described CR as cut-off value to determine whether there was a significant increase of R_{int} during methacholine provocation testing, R_{int} was able to detect only half of the responders as determined by a significant fall in FEV_1. When we evaluated exhalation times in exhaled Nitric Oxide measurements, we concluded that the found difference between values obtained during six and ten seconds exhalation was not clinically relevant, since the measured difference was similar to the difference between repeated measurements.

Reversibility after inhalation of a bronchodilator is one of the characteristics of asthma. The change in lung function is also used in reversibility testing. When FEV_1 increases with ≥12% of the pre-bronchodilator value, reversibility of lung function is thought to be present. Since absolute pre-bronchodilator and post-bronchodilator values are compared, the new reference values will not influence the decision whether reversibility if present or not.

Preschoolers

Until recently, possibilities to measure lung function in young children were scarce. Conventional lung function tests require active participation and therefore infants and preschoolers have often difficulties performing such tests. In the previous years, adult lung function equipment and measurement protocols were accustomed for use in children and new tests were developed to measure lung function in infants and

preschoolers. Although lung function tests for infants are mainly used in research settings, specialised and adjusted lung function tests for children aged 4 to 6 years (preschoolers) are increasingly available. Thirty years ago, when the mother of the girl in case 2 was suffering from wheezing complaints, it was hardly possible to perform lung function tests in preschool children.
Since most children below the age of 4 are not able to perform a forced flow/volume measurement, R_{int} could have been an alternative to detect bronchial obstruction during methacholine provocation tests. However, we showed that R_{int} poorly discriminated between children who did or did not reach the FEV_1 endpoint, impeding detection of bronchial hyperresponsiveness with a measurement of interrupter resistance in the youngest children. Therefore, bronchial hyperresponsiveness could not already have been objectified at the age of three, as questioned by the mother in case 2.
We showed that adjustments to lung function tests could results in higher success rates, since younger children preferred a shorter exhalation time in FeNO measurement. A six seconds exhalation showed a good correlation with the standard exhalation of ten seconds. Without this knowledge, we would not have measured FeNO in the girl in case 2. We recommend the use of a six seconds exhalation in FeNO measurements in young children or children with a restrictive lung disease. This will lead to more children in whom a measure of airway inflammation will be available in diagnosis and follow-up, and more children in whom the effect of inhaled cortical steroids therapy can be evaluated by using FeNO as an outcome measure, in clinical decision making as well as in clinical trials.

Implications for future research

It is obvious that lung function changes over time and reference values should be updated frequently. Future reference data studies might focus on different aspects. Since ethnic differences in lung function exist[26], ideally ethnic specific reference equations should be generated. However, since populations increasingly consist of mixed ethnic groups, it is difficult to define an individual as a member of a specific ethnic group. Furthermore, since environmental factors also influence lung function, not only someone's genetic background, but also the environment influences growth, lung growth and lung development. Therefore, the lung function of a non-Caucasian child living in Western-Europe could be influenced by both negative and positive environmental factors, that could have been absent in the country where its parents were born. When these environmental factors (e.g. health care, diet and air pollution) have more influence on lung function than genetic predisposition, then it is more important to create region specific reference equations rather than ethnic specific values. Future studies should therefore investigate whether reference values, such

as those in this thesis that are based on European Caucasians could for example be applied to European children of a different ethnic descent.
Furthermore, although the ATS and ERS recommend selecting a healthy population to develop reference values, one could argue that for some diseases, disease-specific reference values would be more appropriate. For example in children with cystic fibrosis, a decline in lung function is seen with increasing age. Additionally, the height of these children is reduced compared to same age healthy controls and the start of puberty is delayed as well[25]. Cystic Fibrosis-specific lung function reference values might therefore be more sensitive to detect a atypical decline in lung function in this group of patients.
Another objective of future research is to create new adult reference values for lung function, based on the present adult population, protocols and equipment, since the new paediatric reference values and adult lung function reference equations do not smoothly connect. The best would be to model a complete dataset of new reference data collected in both children and adults, in order to precisely predict the course of lung growth in children and the decline in lung function during adulthood once peak lung function has been reached. The GAMLSS statistical method uses cubic splines to model the sudden change from rising to declining lung function.
Since new lung function techniques are still being developed, those techniques require reference values as well. For example, in children with Cystic Fibrosis the multiple breath wash out measurement is proposed as an alternative for spirometry in young children, in whom flow-volume measurements lack the ability to detect lung function reductions. Therefore, reference values for the lung clearing index should be generated.
Furthermore, future studies should aim to validate paediatric lung function testing, especially in preschoolers, and evaluate the usefulness in clinical decision making.
Ideally, future research should focus on the development of one single lung function measurement that could be used in early infancy, childhood and adulthood. Such an all-age lung function measurement could help to detect lung function reductions early in life and will enable to make comparisons of lung function over time, using the same parameters. An all-age lung function test will overcome the existing problems of comparing different parameters at different ages, expressing different characteristics of the respiratory system. This will aid in follow-up of children with respiratory diseases as well as in longitudinal studies evaluating tracking and detracking of lung growth.

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CHAPTER 10

Summary & Samenvatting

Summary

Paediatric lung function tests provide objective measures in diagnosis and follow-up of lung diseases, and give insight in the pathophysiology of lung growth and development. In this thesis, paediatric lung function tests were used to investigate the influence of familial aggregation, normal and excessive body growth and tobacco smoke exposure on lung growth and development. The second part focussed on the use of paediatric lung function testing and reference values in clinical practice.

Determinants of lung growth and development

In **chapter 2** we investigated the relation between parental lung function and their offspring's neonatal lung function and questioned whether this relation is genetically determined or due to shared environment. In the prospective birth cohort study WHISTLER we measured infant's lung function within 8 weeks after birth, using the single occlusion technique, and compared it to their parents lung function, measured with forced flow volume measurements. There was a significant positive relation between parental FEF_{25-75} and neonatal C_{RS} as well as a significant negative relation between parental FEV_1 and FEF_{25-75} and their child's R_{RS}. After correction for familial aggregation of body growth, as well as shared environmental factors, the relations stayed significant and we concluded that parental lung function partially determines the innate lung function level of their offspring.

Chapter 3 addressed the influence of rapid weight gain in the first three months after birth on respiratory complaints in the first years of life and lung function at the age of five. In the WHISTLER cohort, parents recorded weight and length measurements as well as respiratory symptoms on monthly questionnaires during the first year of life. Data on primary care visits during the first years of life were obtained from the general practitioners' electronic patient files. Forced flow-volume measurements were performed during follow-up at the age of five. We found a significant positive association between accelerated weight gain during the first three months of life and more wheezing symptoms and primary care visits for respiratory symptoms in the first years of life. At the age of five, FEV_1 and FEF_{25-75} were significantly reduced with 2.8% and 5.3% with every standard deviation more weight gain during the first three months of life. We concluded that accelerated weight gain in the first three months of life is a risk factor for clinically relevant wheezing illnesses in the first years of life and lower lung function at school age.

In **chapter 4** the effect of incremental implementation of smoke-free legislation on exposure to environmental tobacco smoke during pregnancy and birth weight and neonatal lung function was determined. In the WHISTLER cohort we showed that

during the previous 10 years during which smoking bans were increasingly introduced in the Netherlands, the percentage of pregnant women that reported smoke exposure during pregnancy significantly decreased from 24.8% in the 2001-2004 group to 5.5% in the 2008-2010 group. After adjustment for potential confounders in the respective groups mean birth weight significantly increase. In the same period there was a significant increase in neonatal lung function, that remained after adjustment for possible confounders. Besides the well-known beneficial effect of smoke-free legislation on lung function of directly exposed persons, this study adds that diminished foetal ETS exposure has a beneficial effect on lung function in newborns as well. Since prenatal smoke exposure is associated with several other health risks, the implementation of smoke-free legislation will probably result in more than only respiratory benefits in newborns.

Chapter 5 concerned the question whether prenatal smoke exposure has a permanent or a temporarily effect on lung function in early childhood. In 225 children from the WHISTLER cohort, the neonatal lung function measurements and the follow-up lung function measurements at the age of five were used to calculate the change in lung function since birth, expressed as change in z-score. Prenatal smoke exposure resulted in decreased neonatal lung function and an increase in lung function when children were not postnatally exposed to tobacco smoke. Postnatal smoke exposure resulted in a lung function decline. We concluded that the negative effects of prenatal smoke exposure wane during early childhood but this waning effect is less in children who are also postnatally exposed to tobacco smoke. Since most prenatally exposed children will also be postnatally exposed, they will still have reduced lung function at the age of five. Therefore, both smoke exposure during pregnancy as well as postnatal smoke exposure should be discouraged to protect children's lung function.

Paediatric lung function measurements

In **chapter 6A** new reference values for the most commonly used lung function tests in children are provided. Because of secular trends in body growth, modifications in measurement protocols, improvement in measurement techniques and progress in statistical analyses, previously published reference equations might be inappropriate. Therefore, there is an ongoing need to generate new reference data for lung function tests. In the 'Utrecht pulmonary function reference data study' we collected data in healthy Caucasian children between 2 and 18 years old. We used the statistical analysis method 'GAMLSS' to analyse the data. We provide reference values for interrupter resistance, spirometry, bodyplethysmography and carbon monoxide diffusion and helium dilution tests. These new reference values account for the coefficient of variation as well as the skewness of the data, and therefore offer a

accurate estimates of the range of normality. The use of these reference values will result in lees underdiagnosis and overdiagnosis of lung diseases.

In **chapter 6B** the spirometry dataset of the 'Utrecht pulmonary function reference data study' was used to investigate the effect of parental smoking on healthy children's lung function. When one or both parents reported to smoke or have smoked now and/or in the past, the child was categorized as "ever ETS exposed". Children whose parents both reported never to have smoked in the home were considered "never ETS exposed". There were significant reductions of FEF_{75} , FEF_{50} , FEF_{25} and FEF_{25-75} values in "ever ETS exposed" children (minus 9.4%, 7.4%, 5.4% and 8.1%) and also small but significant reductions in FEV_1, $FEV_{0.5}$ and PEF. We concluded that parental smoking results in a marked decline of lung function in healthy children and therefore parents should be discouraged to smoke in the nearness of their child.

In **chapter 7** we questioned whether the interrupter technique could be used in bronchoprovocation testing to diagnose bronchial hyperresponsiveness, a key feature of asthma. A 20% fall in FEV_1 is the primary outcome in bronchoprovocation testing, but since young children are unable to perform flow-volume measurements, the interrupter could be an alternative, not requiring active patient participation. A part of the Combo-study, methacholine challenge tests were performed in children with a history of moderate asthma and bronchial hyperresponsiveness. Changes in interrupter resistance as well as changes in FEV_1 were recorded following increasing doses of methacholine. Although there was a significant increase of mean interrupter resistance values with higher doses of methacholine, individual changes were highly variable during methacholine challenge. There was great overlap in change of interrupter resistance between children who did and did not reach the FEV_1 endpoint. The sensitivity and specificity of R_{int} to detect methacholine induced bronchoconstriction was low, which might limit the use of R_{int} in individual patients.

In **chapter 8** we evaluated whether an exhalation time of six seconds instead of ten seconds could be used in the measurement of fractional exhaled nitric oxide (FeNO) in young children, since ten seconds is often too difficult in young children with small vital capacities. We compared FeNO values measured during six and ten seconds exhalation time and showed good agreement between these measurements, meaning that these measurements can be used interchangeably. The majority of the children with forced vital capacities smaller than 3 litres preferred the shorter six seconds exhalation time.

Chapter 9 provided a general discussion presenting the final conclusions from the separate studies as well as implications for future research and clinical practice.

Samenvatting

Bij de diagnose en het vervolgen van longziekten geven resultaten van longfunctietesten een objectieve weergave van de longfunctie. Daarnaast kunnen longfunctietesten in wetenschappelijk onderzoek gebruikt worden om inzicht te krijgen in normale en afwijkende longgroei en –ontwikkeling. In dit proefschrift werden longfunctietesten gebruikt om de invloed van familiaire factoren, normale en excessieve groei en rookexpositie op longgroei en –ontwikkeling te onderzoeken. Het tweede deel van dit proefschrift richtte zich op het gebruik van longfunctietesten en referentiewaarden van longfunctietesten in de klinische praktijk.

Factoren die invloed hebben op long groei en -ontwikkeling

In **hoofdstuk 2** werd de longfunctie van ouders vergeleken met de longfunctie van hun pasgeboren baby en vroegen we ons af in hoeverre de relatie tussen de longfunctie van ouders en hun kinderen bepaald wordt door dezelfde genetische aanleg of door dezelfde omgevingsfactoren. In het prospectieve geboortecohort-onderzoek WHISTLER werd de longfunctie van pasgeboren baby's gemeten binnen 8 weken na de geboorte, gebruik makend van de 'single occlusion' techniek. Deze longfuncties werden vergeleken met de longfuncties van hun ouders, gemeten middels geforceerde flow-volume metingen. Er was een significante positieve relatie tussen de FEF_{25-75} van ouders en de C_{RS} waardes van de pasgeborenen. Tegelijkertijd was er ook een significante negatieve relatie tussen de FEV_1 en FEF_{25-75} waardes van ouders en de R_{RS} waardes van hun kinderen. Na correctie voor familiaire aanleg voor lichaamsgroei en familiaire omgevingsfactoren bleven de relaties tussen longfunctie waarden van ouders en hun pasgeborenen bestaan. We concludeerden dat de longfunctioe van ouders op zijn minst gedeeltelijk de longfunctie van hun kinderen bepaalt.

In **hoofdstuk 3** wordt de invloed van snelle gewichtstoename in de eerste drie levensmaanden op luchtwegklachten in de eerste levensjaren en de longfunctie op vijfjarige leeftijd beschreven. In het WHISTLER cohort noteerden ouders maandelijks de lengte en het gewicht van hun kind. Daarnaast vulden zij in het eerste levensjaar maandelijks vragenlijsten in over luchtwegklachten bij hun kind. Gegevens over huisartsbezoek voor luchtwegklachten in de eerste levensjaren werden verzameld uit de databestanden van de samenwerkende huisartsen. Op de leeftijd van vijf jaar werd de longfunctie van de kinderen gemeten middels een geforceerde flow-volume meting. We vonden een significante positieve relatie tussen versnelde gewichtstoename in de eerste drie maanden na de geboorte en meer piepende luchtwegklachten en huisartsbezoek voor luchtwegklachten in de eerste levensjaren. Op vijfjarige

leeftijd waren FEV_1 en FEF_{25-75} significant afgenomen met 2.8% en 5.3% voor iedere standaarddeviatie meer gewichtstoename in de eerste drie levensmaanden. We concludeerden dat versnelde gewichtstoename in de eerste drie levensmaanden een risicofactor is voor klinische relevant piepen in de eerste levensjaren en een lagere longfunctie op de kleuterleeftijd.

In **hoofdstuk 4** werd het effect van gelijkmatige invoering van de tabakswet op rookexpositie tijdens de zwangerschap onderzocht, alsmede het effect hiervan op het geboortegewicht en de longfunctie van pasgeborenen. In het WHISTLER cohort lieten we zien dat in de afgelopen tien jaren,waarin geleidelijk de tabakswet werd uitgebreid, het percentage zwangere vrouwen dat rapporteerde aan rook te zijn blootgesteld daalde van 24.8% tussen 2001 en 2004 naar 5.5% tussen 2008 en 2010. Na correctie voor mogelijke confounders nam het geboortegewicht significant toe in de opeenvolgende jaargroepen. In dezelfde jaargroepen was er een significante stijging van de longfunctie van pasgeborenen, die bleef bestaan na correctie voor eventuele confounders. Naast het bekende effect van wetgeving die meeroken limiteert op de longfunctie van mensen die direct aan rook worden blootgesteld, laat dit onderzoek nu ook zien dat verminderde blootstelling aan tabaksrook in de baarmoeder ook een voordelig effect heeft op de longfunctie van pasgeborenen. Aangezien rookblootstelling in de baarmoeder geassocieerd is met verscheidene andere gezondheidsrisico's zal de invoer van rookvrij-wetgeving waarschijnlijk resulteren in meer dan alleen voordelen voor de luchtwegen van pasgeborenen.

Hoofdstuk 5 betrof de vraag of blootstelling aan rook in de baarmoeder een permanent of een tijdelijk effect heeft op de longfunctie. Van 225 kinderen uit het WHISTLER cohort werd de longfunctiemeting kort na de geboorte vergeleken met de longfunctiemeting op vijfjarige leeftijd en werd de longfunctie verandering uitgedrukt als het verschil tussen de z-scores van deze metingen. Rookblootstelling in de baarmoeder leidde tot een verlaagde longfunctie kort na de geboorte, gevolgd door een toename van longfunctie gedurende de eerste vijf levensjaren wanneer kinderen na de geboorte niet meer regelmatig aan rook werden blootgesteld. Wanneer kinderen alleen na de geboorte maar niet tijdens ze zwangerschap aan rook werden blootgesteld, resulteerde dit in het dalen van de longfunctie tussen de geboorte en het vijfde levensjaar. We concludeerden dat de negatieve effecten van blootstelling aan rook in de baarmoeder uitdoven wanneer kinderen na de geboorte niet meer aan rook worden blootgesteld. Aangezien de meeste kinderen die in de baarmoeder aan rook werden blootgesteld ook na de geboorte aan rook zullen worden blootgesteld, zal het uitdoven van het negatieve effect maar in een kleine groep kinderen plaatsvinden, en zal het merendeel van de kinderen die in de baarmoeder al aan rook werden

blootgesteld alsnog een verlaagde longfunctie hebben op vijfjarige leeftijd. Daarom blijft het belangrijk om zowel rookblootstelling in de baarmoeder als na de geboorte te ontmoedigen zodat de longfunctie van kinderen beschermd wordt tegen het schadelijke effect van meeroken.

Longfunctiemetingen bij kinderen

In **hoofdstuk 6A** werden nieuwe referentiewaarden gepresenteerd voor de meest gebruikte longfunctietesten op de kinderleeftijd. Omdat er door de jaren heen veranderingen optreden in lichaamsgroei, protocollen voor longfunctietesten, longfunctieapparatuur en statistische analysetechnieken kunnen eerder gebruikte referentiewaarden niet meer geschikt zijn voor de huidige populatie kinderen. Er is dan ook een aanhoudende behoefte aan nieuwe referentiewaarden voor longfunctietesten. In de 'Utrecht pulmonary function reference data study' hebben we longfunctiegegevens verzameld in Caucasische kinderen in de leeftijd van 2 tot 18 jaar. Voor de analyses van deze data hebben we gebruik gemaakt van de statistische methode 'GAMLSS'. We presenteerden nieuwe referentiewaarden voor de volgende longfunctie testen: interrupter weerstand, spirometrie, lichaamsplethysmografie en koolstofmonoxide diffusie- en helium dilutietesten. Deze nieuwe referentiewaarden houden rekening met de variatie en scheve verdeling van longfunctiewaarden in de bevolking en verschaffen daardoor een nauwkeurige schatting van wat nog wel als normaal beschouwd mag worden, en wat afwijkend is. Het gebruik van deze referentiewaarden zal tot minder over- en onderschatting van longziekten leiden.

In **hoofdstuk 6B** werd de spirometrie dataset van de 'Utrecht pulmonary function reference data study' gebruikt om te onderzoeken in hoeverre het roken van ouders effect heeft op de longfunctie van hun kinderen. Wanneer een of beide ouders rapporteerden dat ze nu of in het verleden in huis hadden gerookt werd hun kind gecategoriseerd als "ooit aan rook blootgesteld". Wanneer ouders rapporteerden dat zij beide nooit in huis hadden gerookt, werd hun kind gecategoriseerd als "nooit aan rook blootgesteld". De "ooit aan rook blootgestelde" kinderen hadden een significant verlaagde longfunctie. FEF_{75} , FEF_{50} , FEF_{25} and FEF_{25-75} waarden waren met respectievelijk 9.4%, 7.4%, 5.4% en 8.1% verlaagd en er werden ook kleine maar significante verlagingen van FEV_1, $FEV_{0.5}$ and PEF gezien. We concludeerden dat roken door ouders tot een duidelijke afname van de longfunctie van kinderen leidt en dat ouders moeten worden aangespoord om niet te roken in de nabijheid van hun kind.

Bronchiale provocatietesten worden gebruikt om bronchiale hyperreactiviteit vast te stellen. Dit is een van de kenmerken van astma. De primaire uitkomstmaat van deze testen is een 20% verlaging van de FEV_1 waarde. Omdat jonge kinderen

niet voldoende kunnen meewerken om een goede FEV_1 te blazen, zou er voor die kinderen een alternatief moeten zijn om bronchiale hyperreactiviteit te objectiveren. In **hoofdstuk 7** vroegen we ons af of de interrupter techniek gebruikt zou kunnen worden bij bronchiale provocatietesten in jonge kinderen. Als onderdeel van de Combo-studie werden methacholine provocatietesten uitgevoerd door kinderen met mild astma en bronchiale hyperreactiviteit. Veranderingen in de interrupter weerstand en de FEV_1 werden vastgelegd na stapsgewijs toedienen van opklimmende doses methacholine. Op groepsniveau was er gemiddeld een significante toename van de interrupter weerstand bij opklimmende hoeveelheden ingeademd methacholine. Daarentegen lieten de individuele interrupter waarden zeer veel variatie zien en werden stijgingen vaak weer gevolgd door dalingen. Daarnaast was er een grote overlap van de gemeten verandering in interrupter weerstand tussen kinderen die wel en niet een 20% afname van de FEV_1 lieten zien. De sensitiviteit en specificiteit van interrupter weerstand om bronchiale hyperreactiviteit aan te tonen was laag, wat het nut van deze test in individuele patiënten beperkt.

In **hoofdstuk 8** werd gekeken of de uitademingtijd bij stikstofmonoxide metingen verkort kon worden van tien naar zes seconden, zodat deze test ook uitgevoerd zou kunnen worden in kleiner kinderen met een kleinere longinhoud. We vergeleken metingen verkregen tijdens een uitademing van zes seconden met die verkregen tijdens de conventionele tien seconden uitademingtijd. Deze waarden kwamen goed met elkaar overeen, wat betekent dat de uitslagen van zes en tien seconden metingen met elkaar vergeleken kunnen worden. De meerderheid van de kinderen met een longinhoud van minder dan 3 liter gaven aan dat ze de zes seconden meting makkelijker uitvoerbaar vonden dan de tien seconden meting.

Hoofdstuk 9 geeft een algemene discussie waarin de conclusies van de verschillende studies worden samengevat en wordt ingegaan op wat dit betekent voor vervolgonderzoek en de dagelijkse praktijk van longfunctiemetingen bij kinderen.

Dankwoord

Curriculum Vitae

List of publications

List of abbreviations

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Inka, David, Mats, Bina en Kaush: A Dutch proverb: "It's better to have a good neighbour than a distant friend". Sometimes, Dutch proverbs are wrong. Thanks for our great weekends!

Wilfred, bedankt voor je wetenschappelijke adviezen in de skilift! Manon, er zijn weinig mensen die net zo goed kunnen kletsen als ik. Ik voel me heel erg thuis bij jullie, en heb altijd heel veel steun en gezelligheid ervaren. Veel dank! Sophie, Olivier en Pippa, jullie zullen niet begrepen hebben hoe belangrijk jullie afleiding vaak voor me was. Ik hoop binnenkort weer lekker met z'n allen van de berg af te skiën. Nog even en dan halen jullie me in… Ik ben zo trots op jullie!

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jy kent my skaduwekant
jy weet hoe ek kan wees
jy het my weer laat sing
jy het my stem gegee
(naar Stef Bos)

Curriculum Vitae

Marije Koopman werd geboren op 20 november 1979 te Middelburg. In 1997 behaalde zij haar VWO-diploma aan de Stedelijke Scholengemeenschap Middelburg, waarna zij voor een jaar naar Brussel vertrok om daar als au pair te werken. Voordat zij de studie Geneeskunde startte aan de Universiteit Maastricht, studeerde zij een jaar Gezondheidswetenschappen aan dezelfde universiteit. Tijdens haar studie Geneeskunde liep zij buitenlandse stages in het Booth Hall Children's Hospital in Manchester (Verenigd Koninkrijk), het Hospital Universitario Salamanca (Spanje) en het Hospital Rural de Yaruqui (Ecuador). Haar onderzoeksstage voerde zij uit onder leiding van Prof. Dr. Alexander Ferguson op de afdeling kinderallergologie van het British Columbia's Children's Hospital te Vancouver (Canada). Na haar afstuderen in augustus 2005 (cum laude) was zij een jaar werkzaam als arts-assistent kindergeneeskunde in het Sint Elisabeth Ziekenhuis te Tilburg (opleider Dr. Pim van Dijken; thans Dr. Charlie Obihara). In oktober 2006 startte zij haar promotieonderzoek op de afdeling Kinderlongziekten van het Wilhelmina Kinderziekenhuis te Utrecht, onder begeleiding van haar promotor Prof. Dr. Kors van der Ent en haar co-promotoren Dr. Cuno Uiterwaal en Dr. Bert Arets. Tijdens haar promotieonderzoek volgde zij de Master Epidemiologie, specialisatie Klinische Epidemiologie, aan de Universiteit Utrecht, waarvan zij in september 2010 het diploma behaalde. Sinds juli 2010 is zij in opleiding tot kinderarts in het Wilhelmina Kinderziekenhuis te Utrecht (opleider Dr. Joost Frenkel). In het kader van haar perifere stage werkt zij momenteel met veel plezier in het Ziekenhuis Gelderse Vallei te Ede (opleider Dr. Gert van Enk).

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M.M. van der Zalm, C.S.P.M. Uiterwaal, B. Wilbrink, J.W.A van Rossen, M. Koopman, T.J.M. Verheij, C.K. van der Ent. The association between respiratory symptoms and the presence of respiratory pathogens in infants during the first year of life. *Submitted.*

List of abbreviations

ATS	American Thoracic Society
AUC	Area under the curve
BHR	Bronchial hyperresponsiveness
BTPS	Body temperature and pressure saturated
CI	Confidence interval
CO	Carbon monoxide
CR	Coefficient of repeatability
C_{RS}	Compliance of the total respiratory system
CV	Coefficient of variation
Delta-Z-FEF_{25-75} -C_{RS}	Difference in Z-FEV_1 and Z-C_{RS}
Delta-Z-FEF_{25-75} -R_{RS}	Difference in Z-FEF_{25-75} and negative value of Z-R_{RS}
Delta-Z-FEV_1-C_{RS}	Difference in Z-FEV_1 and Z-C_{RS}
Delta-Z-FEV_1-R_{RS}	Difference in Z-FEV_1 and negative value of Z-R_{RS}
$D_{L,CO}$	Single breath CO diffusion capacity
ERS	European Respiratory Society
ERV	Expiratory residual volume
ETS	Environmental tobacco smoke
FEF_{25}	Maximal expiratory flow when 25% of FVC is expired
FEF_{25-75}	Forced expiratory flow between 25% and 75% of FVC
FEF_{50}	Maximal expiratory flow when 50% of FVC is expired
FEF_{75}	Maximal expiratory flow when 75% of FVC is expired
FeNO	Fractional exhaled nitric oxide
FeNO-10	FeNO level during 10 seconds exhalation
FeNO-6	FeNO level during 6 seconds exhalation
FEV_1	Forced expiratory volume in 1 second
FRC	Functional residual capacity
FVC	Forced vital capacity
GAMLSS	Generalized additive models for location scale and shape
GINA	Global Initiative for Asthma
$_{He}$	Measured with He dilution
He	Helium
ICPC	International Classification of Primary Care

IQR	Interquartile range
IRR	Incidence rate ratio
IVC	Inspiratory vital capacity
LLN	Lower limit of normal; 5th percentile
LRGP	Leidsche Rijn GezondheidsProject
n	Number
NO	Nitric oxide
p	P-value
PD_{20}	20% fall in FEV_1
$PD_{20}P_{tc}O_2$	20% fall in transcutaneous partial pressure of oxygen
$_{pleth}$	Measured with body plethysmography
$Raw_{0.5}$	Airway resistance at inspiratory and expiratory flows of 0.5 L/s
Raw_{tot}	Total airway resistance
R_{int}	Interrupter resistance
ROC	Receiver operating characteristic
R_{RS}	Resistance of the total respiratory system
RV	Residual volume
SBOL	Single-breath online technique
SD	Standard deviation
SES	Socio-economic status
SOT	Single occlusion technique
TLC	Total lung capacity
T_{RS}	Time constant of the total respiratory system
ULN	Upper limit of normal; 95th percentile
VA	Alveolar volume
VT	Tidal volume
WHISTLER	WHeezing Illnesses STudy LEidsche Rijn
WHO	World Health Organisation
Z-C_{RS}	C_{RS} z-score
Z-FEF_{25-75}	FEF_{25-75} z-score
Z-FEV_1	FEV_1 z-score
Z-R_{RS}	R_{RS} z-score

Waarom niet

Aan het einde
Kunnen zeggen
Ik weet het niet
Met de rust
Dat te weten

Stef Bos