Shared early origins of cardiovascular and respiratory development

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te Steenwijk

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Contents

Chapter 1

General introduction

Non-communicable diseases

Non-communicable diseases, like diabetes mellitus, chronic respiratory and cardiovascular diseases, are the major causes of death and disability worldwide and the prevalence of these diseases are expected to increase substantially in the future.[1] Of the 57 million global deaths in 2008, 63 million (63%) were due to non-communicable diseases. Of these deaths, 60% could be attributed to cardiovascular and respiratory diseases.

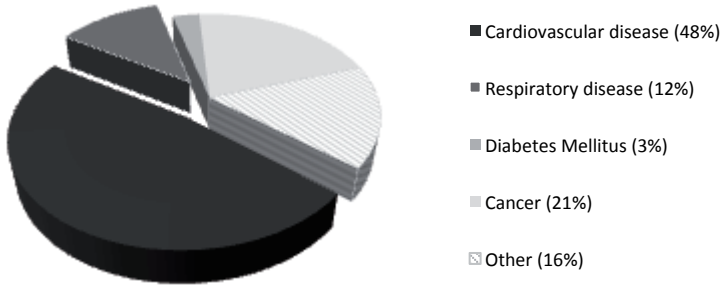

Figure 1. *Distribution of global non-communicable disease by cause of death (WHO 2008)*

The World Economic Forum placed chronic diseases in the top 5 leading threats obstructing economic development.[2] With better treatment options and an increasing and aging population, healthcare costs are rising, while productivity and economic growth are reduced, as half of those who die from chronic diseases are in their productive years.[2]

Risk factors of non-communicable chronic diseases

There is much overlap in risk factors for the different types of chronic disease. The main risk factors of non-communicable disease are shown in figure 2. Morbidity and mortality from chronic diseases are largely preventable through the reduction of these risk factors. Globally, smoking causes 10% of cardiovascular disease and 42% of chronic respiratory disease. Of all global deaths, 9% are attributed to smoking.[3] Other risk factors like raised blood pressure attribute for 13%, raised blood glucose 6%, physical inactivity 6% and 5% to overweight and obesity.[3]

Coexistence of cardiovascular and respiratory diseases

Chronic diseases are often studied separately, but there is growing awareness that these diseases are closely linked.[4] Various chronic diseases often coexist within individuals. Cohort studies in adults described associations between chronic obstructive pulmonary disease (COPD) and cardiovascular disease.[5] In patients with mild-to-moderate COPD cardiovascular disease is even the most important cause of mortality.[6] Lung function and precursors of cardiovascular diseases, like high blood pressure and arterial stiffness have been associated as well.[7–12]

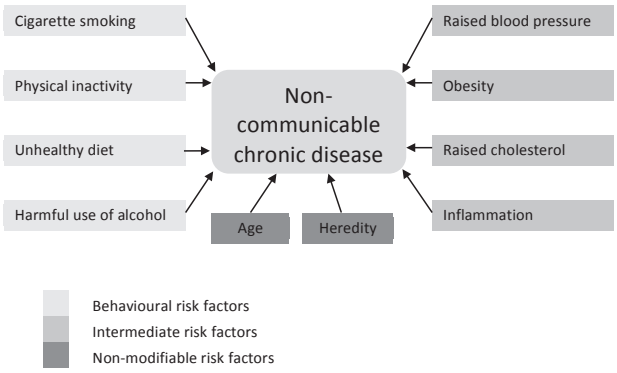

Figure 2. *Main risk factors of non-communicable disease*

In the last decade strong epidemiological evidence indicates that poor lung function is a risk factor for the development of cardiovascular disease.[5, 13] The complex nature of interactions between the cardiovascular and respiratory system is far from understood. The extent to which associations are due to common disease mechanisms or whether shared risk factors are involved is unknown. However, major risk factors for cardiovascular and respiratory diseases, like cigarette smoking, age and chronic inflammation, could only partially explain these associations.[9, 11, 14]

Developmental origins of chronic diseases

Along with genetic predisposition and lifestyle, there is increasing evidence for another factor that contributes to the development of chronic diseases, the intra-uterine and postnatal environment.[15] Early life adverse exposures could induce changes in development with a long-term impact on later health and disease risk, which is also known as the Developmental Origins of Health and Disease (DOHAD) hypothesis. Many of the non-communicable diseases appear to have their origins in utero or in early childhood. Extensive epidemiological evidence is derived from studies that describe associations between low birth weight, as a marker of fetal under-nutrition, and the development of chronic diseases, including cardiovascular and respiratory disease.[16–18] Other early exposures of interest include maternal smoking and diet, infant feeding and infectious diseases in early life. [19] Longitudinal follow-up studies have shown that risk factors for cardiovascular disease and respiratory disease in childhood, like blood pressure and lung function track into adulthood.[20–22] Therefore, impaired cardiovascular or lung development in early life, could predispose the individual to respiratory or cardiovascular disease in later life. It is also known that health behaviour, acquired in childhood, continues into adulthood. Altogether, this suggests that prevention of the development of risk factors in early life (primordial prevention) could be an effective way to reduce the development of chronic disease in later life. Much research is performed to study early origins of a single chronic disease, but only little is known about the link between the development of chronic diseases and shared risk factors in early life. Studies focused on this topic might extend our knowledge on the origin of the link between chronic diseases.

Objective of this thesis

In this thesis the developmental origins of cardiovascular and respiratory diseases are studied. The general aim of this thesis was to study shared risk factors and the interaction between the cardiovascular and respiratory system in early life.

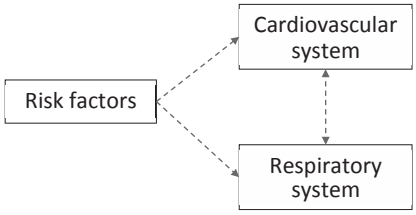

Figure 3. *Associations studied in this thesis*

Outline of this thesis

The next three chapters describe the interaction of the cardiovascular and respiratory system in early life. In **chapter 2** the relation between blood pressure and lung function from neonatal to elderly age is studied. **Chapter 3** describes the relation between arterial stiffness and lung function in young children. In children with a disease which is characterized by severe pulmonary problems, cystic fibrosis, we studied arterial stiffness and cardiac function. These results are presented in **chapter 4.**

Weight is an important modifiable risk factor for both respiratory and cardiovascular disease. In **chapter 5 and 6** we studied the association between maternal relative weight and the cardiovascular and respiratory system, respectively. A cross-sectional association at age 8 between leptin and lung function is described in **chapter 7**.

Hard end points of chronic cardiovascular and respiratory disease only will become apparent in adulthood. Non-invasive methods to accurately measure the cardiovascular and respiratory system in childhood will improve our ability to risk-stratify young individuals. Assessment of these systems in early childhood is challenging. In the last part of this thesis, we studied new non-invasive measurement devices which have the potential to evaluate cardiovascular and respiratory risk in youth. **Chapter 8** describes the methodological aspects and main determinants of measurement of pulse wave velocity, an indirect marker of arterial stiffness in young childhood. **Chapter 9** presents the results of measurements with the Wholter, a device which measures wheezing, a common respiratory symptom in childhood. Determinants of lung function and feasibility of measurements of respiratory compliance and resistance in healthy Southeast Asian infants are described in **chapter 10**.

Finally, **chapter 11** provides a general discussion with our main findings and implications for future research, which is followed by a summary in **chapter 12**.

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Part I

Interaction of the cardiovascular and respiratory system in childhood

Chapter 2

Life-course of cardio-respiratory associations

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Eur J Prev Cardiol. 2013 Oct 30.

Abstract

Background

Several studies showed that raised cardiovascular risk factors are associated with an impaired lung function in adulthood. Whether this association also exists in the young is unknown. Our aim was to study the relation between blood pressure and lung function from neonatal to elderly age. This cross-sectional study is performed in a general population cohort.

Methods

Within the Utrecht Health Project (UHP) 6673 adults (18-91 year) had spirometry and blood pressure measurements. In the WHISTLER study, a satellite birth cohort of the UHP, blood pressure and respiratory mechanics were measured, using the single occlusion technique in 755 newborns and spirometry in 382 5-year-old participants. Linear regression analyses were performed with lung function as independent variable and blood pressure as dependent variable in different age groups. The analyses were adjusted for age, sex, weight and height.

Results

In infancy a more favourable lung function (higher compliance and lower resistance) was associated with higher blood pressure. In 5-year-olds and young adulthood higher FEV1 was associated with higher systolic blood pressure (p-values <0.05). At the age of 5 the adjusted regression coefficient for systolic blood pressure was 4.8 mmHg/L (95% confidence interval (95% CI) 0.3- 10.0). The association decreased with increasing age and reversed in the age groups above 40 years to -7.3 mmHg/L (95% CI -15.5 - 0.9) in >70 year olds. The association with pulse pressure showed a similar pattern.

Conclusion

A positive association between mechanical properties of the respiratory system and blood pressure in childhood and young adulthood reverses in later adulthood.

Introduction

Interactions between the pulmonary and cardiovascular systems are increasingly becoming a research focus.[1, 2] In adults, diseases of both systems often co-exist, which might suggest shared origins. In patients with mild to moderate chronic obstructive pulmonary disease (COPD), cardiovascular disease is the most common comorbidity and leading cause of hospitalization.[3, 4] A recent study showed that individuals with COPD had a twofold higher risk of carotid artery wall thickening on ultrasonography than control subjects with normal lung function. This risk was significantly higher with more severe airflow limitation.[5] There are also other observations in adults showing that impaired lung function is related to cardiovascular disease, notably stiffer and thicker arteries and higher blood pressure.[6–9] Major risk factors for both cardiovascular and respiratory disease, like age, cigarette smoking and chronic inflammation, only partially explain the associations.

Over the last decades, there has been growing interest in the early life origins of later life chronic disease. The Developmental Origins of Health And Disease (DOHAD) hypothesis proposes that early life adverse exposures lead to permanent metabolic or structural changes, that in later life result in cardiovascular or respiratory disease.[10,11] Many studies have shown early life origins of cardiovascular disease and respiratory disease separately. However, while these diseases are clearly related in later adulthood, there is only scarce information about whether they might share early life origins. Such knowledge is important, because prevention of major later life chronic diseases need to begin in childhood. Currently, prevention often focuses on single risk factors for separate diseases, while even within diseases a more integrated approach is probably required.[12] If respiratory and vascular disease do share early life origins, it is very likely that prevention in early life will require an even wider scope.

To our knowledge, the only currently available evidence for shared origins in healthy children is that lower forced expiratory volume in 1 second (FEV1) was found related to increased vascular stiffness measured by carotid augmentation index in 8-year-old children.[13]

Further evidence comes mainly from paediatric patients. For instance, in obese children with obstructive sleep apnoea there are already arterial alterations and in children with diabetes mellitus type 1, higher recurrence of infection is related to pre-atherosclerotic change.[14, 15] Existence of such natural relations can best be investigated in healthy young subjects.

Blood pressure is a classical cardiovascular risk factor of which the life-long natural history has been described and is a clear indicator for arterial development from early age onwards.[16] Likewise, lung function measurements are indicators of respiratory diseases at all ages.[17] Both measurements can relatively easily be performed in large-scale population studies and nowadays even in neonates.[18] Our aim was to study the relation between blood pressure and lung function from neonatal to elderly age.

Methods

Setting and participants

The adults are participants of the Utrecht Health Project (UHP), a large health monitoring study of residents of Leidsche Rijn, a newly built residential area near the city of Utrecht.[19] The aim of this study is to create a solid and continuous research infrastructure to generate general medical and health care insights as a basis for evidence-based medicine and health policy. The UHP started to recruit participants in 2001 and invited each new inhabitant, irrespective of age, who registered with a general practitioner by mail to participate. An individual health profile is made for every participant that includes an interview-assisted questionnaire and physical examination. The children are participants of the Wheezing Illnesses STudy LEidsche Rijn (WHISTLER), a satellite cohort of UHP. WHISTLER is a prospective birth cohort study that was initiated in December 2001. Its focus is on early life determinants of respiratory and cardiovascular disease.[18] Lung function, length and weight were measured between 3 and 8 weeks of age, before any respiratory infection occurred. Participants of this study were all born after January 2003, since blood pressure measurements at this visit were started in 2003. At the age of five years, the children who participated in infancy were invited for a second visit. The UHP and WHISTLER studies have been approved by the Medical Ethics Committee of the University Medical Centre Utrecht and conformed to the standards set by the declaration of Helsinki. Written informed consent was obtained from all (or of the parents of) participants.

Measurements

With a self-report questionnaire for adults, smoking status (never, former or current smokers) and a physician's diagnosis of asthma or cardiovascular disease in the past year was assessed. Cardiovascular disease was defined as hypertension, myocardial infarction or stroke in the past year. For children health information was gathered by parental questionnaire. Questions for the newborns included information about smoking of one of the parents and feeding of the child. Questions for the 5-year-old participants included information about a physician's diagnosis of asthma.

Height and weight were measured using a standard electronic scale and body length using an (infant) stadiometer.

Lung function measurements

In infancy, respiratory mechanics were measured using the single occlusion technique during natural sleep according to the guidelines of the European Respiratory Society.[20, 21] At least three technically acceptable flow-volume curves were used to calculate mean resistance and compliance. Lung function measurements were performed by WHISTLER staff. Elaborate interrater reliability studies were performed as part of WHISTLER and showed satisfactory quality of lung function measurements.[21] At the age of five years, lung function was evaluated using a heated Lilly head pneumotachometer system (Viasys Healthcare, Hochberg, Germany) and conform to the latest American Thoracic Society (ATS)/European Respiratory Society (ERS) statement for lung function measurements in preschoolers.[22, 23] At least two

reproducible flow-volume curves were obtained. The largest FEV1 was selected from the curve with highest sum of FEV1 and Forced Vital Capacity (FVC). In adulthood 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 ATS.[23] The maximum of the three measurements was used for analyses.

Blood pressure measurements

Blood pressure measurements in infancy were started in January 2003 in neonates in whom lung function could be successfully measured. Blood pressure measurements were performed during natural sleep three times at the lower leg using an electronic device (DINAMAP; Criticon, model 1846SX). In children at the age of five years and in the adults blood pressure was recorded twice in sitting position at the brachial artery using a semiautomatic oscillometric device (DINAMAP; Criticon, Tampa, FL) in children and an Omron M4 device (Medizintechnik Handelsgesellschaft mbH, Mannheim, Germany) in adults. In all individuals the average of blood pressure measurements was used for analyses.

Data analysis

Central estimators and variance measures to describe general characteristics of the participants were calculated. All variables were checked for normality of distribution and, if necessary, transformations were applied. Adults of UHP were divided into age groups. Differences between age groups were tested using analysis of variance or Chi-square tests whenever appropriate. Linear regression analysis was performed for all associations between lung function and blood pressure. All analyses were adjusted for age, sex, weight and height. In WHISTLER infants compliance and resistance of the respiratory system were independent variables and blood pressure dependent variable, with additional adjustments for smoke exposure of the mother during pregnancy and nutrition of the child (breastfeeding, bottle feeding or a combination). In WHISTLER 5-year olds and adults of UHP, FEV1 was used as independent and blood pressure as dependent variable.

Multivariable models with the product of age and lung function as interaction term were applied to study the trend of the linear regression coefficients. In these models we also adjusted for smoking, asthma and cardiovascular disease. The results are expressed as linear regression coefficients, p-values and 95% confidence intervals (CI). Confidence intervals not including zero and p-values <0.05 were considered statistically significant. All analyses were performed using SPSS for windows, version 17.0.

Results

The baseline characteristics of the 7428 individuals are shown per age group in table 1.

Table 1. *Characteristics per age group (in years)*

		0	5	18-30	31-40	41-50	51-60	61-70	>70	p-value
N		755	382	1662	2817	997	663	394	140	
Male (%)		44.9	46.6	34.2	47.7	49.6	44.3	52.5	55.7	<0.01
Smoking (%)	Yes			17.6	21.4	31.2	26.5	19.3	10.0	
	No			57.9	49.2	31.1	26.5	29.4	33.6	<0.01
	Ever			20.9	25.1	33.2	44.6	49.0	54.3	
Age (years)		0.9 (0.2)	5 (0.3)	27 (3)	35 (3)	45 (3)	55 (3)	65 (3)	76 (4)	<0.01
Height (cm)		22 (2)	115 (5)	174 (10)	175 (10)	173 (10)	171 (9)	170 (9)	167 (9)	<0.01
Weight (kg)		4.5 (0.6)	20.1 (2.8)	73.3 (14.4)	77.8 (15.3)	78.5 (15.8)	78.7 (14.3)	78.1 (13.9)	73.7 (11.5)	<0.01
Lung function										
Compliance (ml/kPa)		45.2 (10.8)								
Resistance (kPa/L/s)		6.8 (2.1)								
FEV1 (L)			1.3 (0.2)	3.6 (0.8)	3.6 (0.9)	3.3 (0.8)	2.9 (0.8)	2.5 (0.7)	2.1 (0.7)	<0.01
PEF ($L \cdot s^{-1}$)			2.8 (0.6)	7.2 (2.2)	7.6 (2.3)	7.1 (2.3)	6.4 (2.2)	5.7 (2.2)	5.0 (2.1)	<0.01
FVC (L)			1.4 (0.2)	4.2 (1.0)	4.3 (1.1)	3.9 (1.0)	3.4 (0.9)	3.0 (0.9)	2.5 (0.8)	<0.01
Blood pressure										
Systolic (mm Hg)		85.6 (10.9)	105.3 (7.9)	120.3 (13.6)	121.1 (14.7)	125.2 (17.0)	134.2 (19.9)	145.3 (21.6)	153.5 (24.1)	<0.01
Diastolic (mm Hg)		38.5 (9.4)	59.1 (9.6)	74.1 (9.0)	75.8 (9.6)	80.0 (10.6)	83.4 (10.8)	84.2 (11.55)	83.0 (10.6)	<0.01
Pulse Pressure (mm Hg)		47.0 (8.8)	50.6 (7.8)	46.2 (10.9)	45.3 (10.5)	45.2 (11.2)	50.8 (14.3)	60.8 (16.1)	70.4 (20.4)	<0.01
MAP (mm Hg)		54.2 (9.0)	71.6 (6.5)	89.5 (9.4)	90.9 (10.4)	95.1 (11.9)	100.4 (12.8)	104.5 (13.6)	106.4 (13.2)	<0.01

FEV1, Forced Expiratory Volume in 1 second; PEF, Peak Expiratory Flow; FVC, Forced Expiratory Volume; MAP Mean Arterial Pressure. Values are means and standard deviations, unless otherwise indicated.

Figures 1 and 2 show the mean systolic and diastolic blood pressure and the mean FEV1 per age group, respectively. Systolic blood pressure increased at young age, stabilized around middle age and further increased in the elderly. Diastolic blood pressure increased at young age, stabilized around middle age, and tended to decrease in the elderly. FEV1 increased in the very young, peaked in young adulthood and then gradually decreased with age.

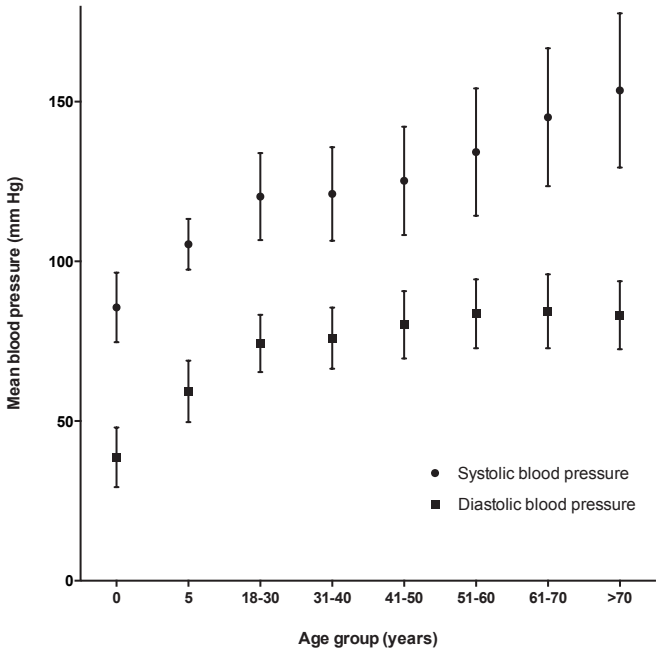

Figure 1. *Mean (standard deviation) systolic and diastolic blood pressure per age group.*

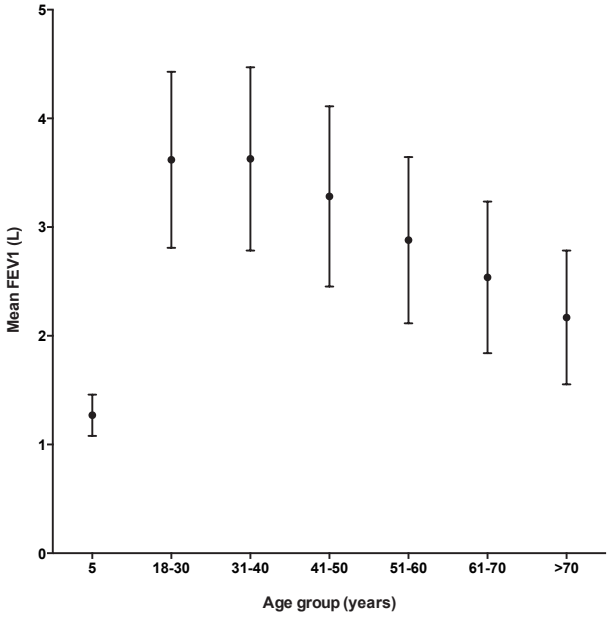

Figure 2. *Mean (standard deviation) FEV1 per age group.*

In 755 newborns we analyzed the associations between lung function as measured by the single occlusion technique and blood pressure. Lung compliance was positively associated with systolic blood pressure (0.18 mm Hg/ml/kPa, 95% confidence interval 0.11 to 0.26, after adjustment for age, sex, length and weight 0.08 mmHg/ml/kPa, 95% CI 0.00 to 0.15) and diastolic blood pressure (0.13 mmHg, 95% CI 0.07 to 0.19, after adjustment 0.09 mmHg/ml/kPa, 95% CI 0.03 to 0.16). Lung resistance was negatively associated with systolic blood pressure (-0.72 mmHg/kPa/L/s, 95% CI -1.08 to -0.35, after adjustment -0.42 mmHg/kPa/L/s, 95% CI -0.80 to -0.05) and with diastolic blood pressure (-0.44 mmHg/kPa/L/s, 95% CI -0.75 to -0.13 and after adjustment: -0.27 mmHg/kPa/L/s, 95% CI -0.60 to 0.06). Adjustment for smoke exposure or nutrition of the child did not influence the associations.

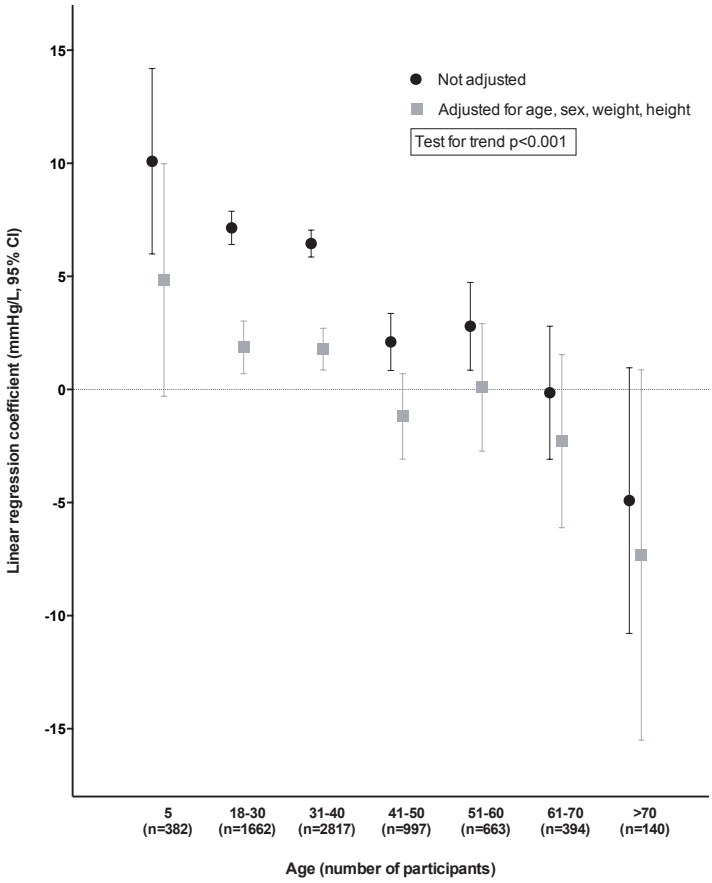

Figure 3. *Associations between systolic blood pressure and FEV1 at different ages.*

Figure 3 shows associations between lung function measured by spirometry (FEV1) and systolic blood pressure from age 5 years onwards. The association was strongest in 5 year olds (4.8 mmHg/L, 95% CI -0.3 to 10.0, adjusted for age, sex, length and weight) and gradually decreased to the weakest in >70 year olds (-7.3 mmHg/L, 95% CI -15.5 to 0.9, adjusted for age, sex, weight and height), while apparently reversing direction between age 50 and 70 years. This decreasing trend of the linear regression coefficients across increasing age was statistically significant (linear regression coefficient of the interaction term: -0.191, p-value <0.001). Adjustment for smoking, asthma or cardiovascular disease had no influence on the associations (linear regression coefficients age-interaction term: -0.189, -0.187 and -0.173 respectively, all with a p-value <0.001).

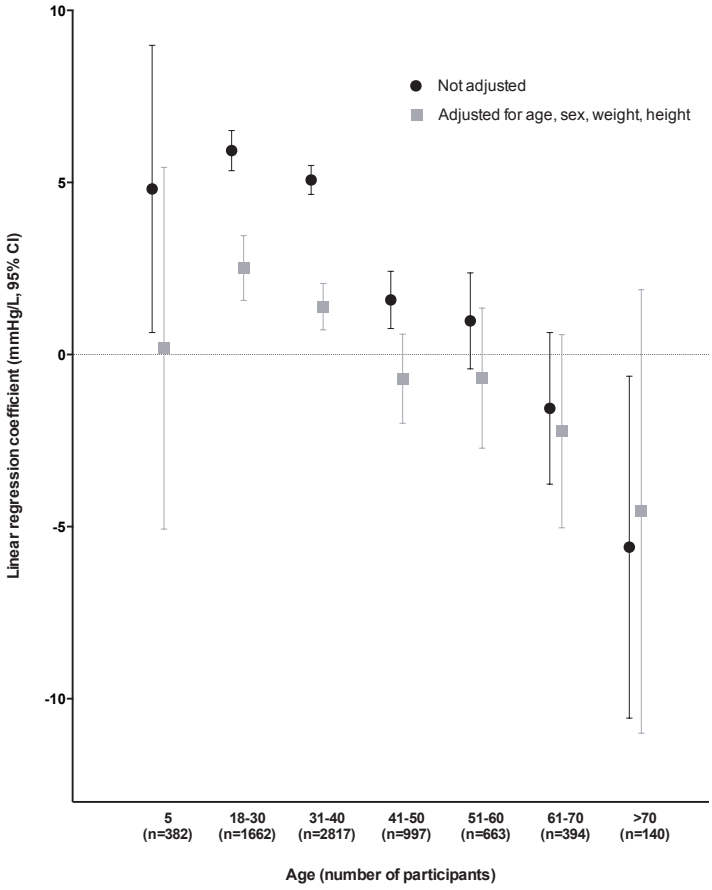

Figure 4. *Associations between pulse pressure and FEV1 at different ages.*

Figure 4 shows similar results for associations between FEV1 and pulse pressure.

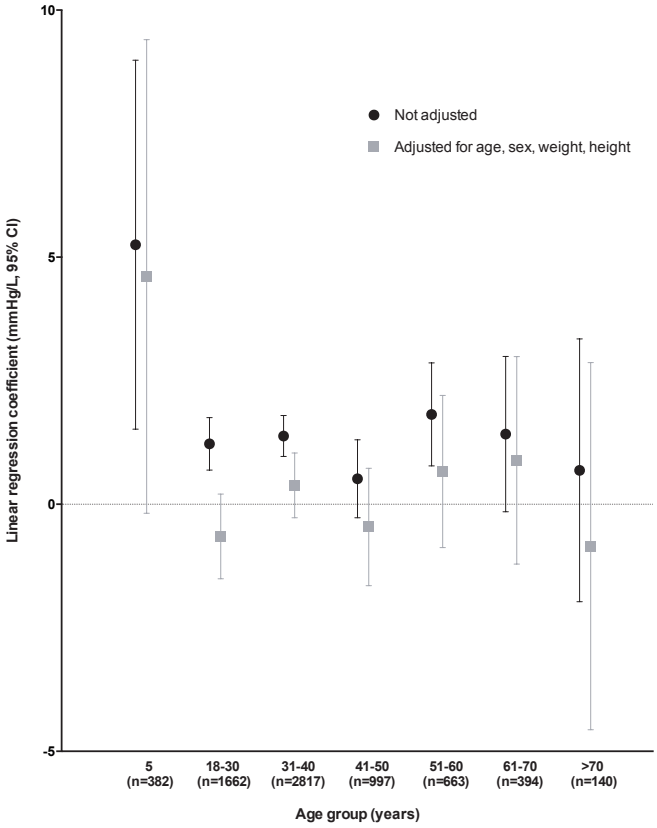

Figure 5. *Associations between diastolic blood pressure and FEV1 at different ages.*

Figure 5 shows associations between FEV1 and diastolic blood pressure. At the age of 5 years a higher FEV1 was associated with a higher diastolic blood pressure (4.6 mmHg/L, 95% CI -0.2 to 9.4, adjusted for age, sex, weight and height). In the older age groups the association remained positive, but not statistically significant after adjustment for age, sex, weight and height.

Discussion

Our study shows that in contrast to findings in elderly, in young individuals lung function and systolic blood pressure show a direct association. This association between mechanical properties of the respiratory system and blood pressure seems to reverse with aging.

To our knowledge, this is the first study investigating the association between blood pressure and lung function in a large unselected population across different ages, including the neonatal period, childhood and early adulthood. It is remarkable that a significant positive association in childhood and

early adulthood was observed. Results are consistent in the early age groups and adjustments for the associations in infancy for smoking of parents, nutrition (breast- or bottle feeding) and heart rate did not influence the results. This finding suggests that a relation between mechanical properties of the respiratory system and systemic blood pressure is part of normal physiology in early life.

Some characteristics of our research need to be considered. To be able to study lung function in early infancy we used the single occlusion technique, while we used spirometry in children and adults. Although we analysed lung function as assessed by two different lung function techniques, it seems reasonable to assume that properties of lung function, including the size of the airways and lungs and the elastic recoil and resistance properties are adequately estimated by both techniques. The direction of the findings in infancy is similar to the findings at the age of 5 years (a lower lung function is associated with higher systolic blood pressure). A further limitation may be that we used cross-sectional analyses. Although we do consider our analyses valid and statistically robust, longitudinal analysis of change of the association within persons over time could possibly give further insight.

The association between blood pressure and lung function has not been studied before in healthy children. A relationship between lung function and arterial stiffness was found in 8-year-old children, with carotid augmentation index being inversely associated with FEV1.[13] As an approximation of arterial stiffness we calculated pulse pressure and found an association in the opposite direction for pulse pressure and FEV1 in childhood and young adulthood. Our findings are more in agreement with cross-sectional findings in adults. In adults higher FEV1 is being associated with lower pulse pressure among those aged ≥ 40 years, but below 40 years an opposite direction was found, although not significant anymore after adjustment for confounders.[9] Our findings in the elderly are also consistent with previous studies.[7, 9, 24, 25]

The reversal of the association after the age of 40 years might reflect several mechanisms. Firstly, it can be speculated that this change is associated with prolonged exposure to pulmonary and cardiovascular disease risk factors, like cigarette smoking and chronic inflammation.[26, 27] Long-term exposure to these factors could lead to an increase in blood pressure and a decline in lung function. In a similar study in adulthood it was demonstrated that the interrelationship between FEV1 and pulse pressure in adults was not fully explained by factors such as smoking, systemic inflammation and other cardiovascular risk factors.[9] In our study, we performed an analysis with adjustment for smoking, which had no effect on the association. In infancy and early childhood these factors are of minor importance. However, the effect of unknown unmeasured factors cannot be entirely ruled out.

A second explanation for the reversal of the cardio respiratory association might be tissue stiffening (aging) of the pulmonary and vascular system over time. Stiffening of respiratory tissue might simultaneously lead to increase of airway resistance and fall in FEV1, as well as to increase of resistance in vascular walls leading to rising blood pressures.

Third, it can be hypothesized that growth plays a role in the decrease of the direct association in childhood and early adulthood. In childhood accelerated weight gain is associated with higher blood pressure and lower lung function.[28, 29] In addition, the natural course of FEV1 and blood pressure, as

shown in figure 1 and 2, shows that in childhood and early adulthood the rise in FEV1 is steeper than the rise in blood pressure. After the age of 40 years the systolic blood pressure still increases, while FEV1 decreases. However, due to the cross-sectional design it is difficult to draw definite conclusions.

Finally, the changes in pulmonary and vascular system over time might be directly causally related. Recently, it was shown in a large longitudinal study that a decline in FVC from average age at peak (29.4 years) to 35 years significantly predicted incidence of arterial hypertension during the 10-year-follow-up. The direction of this association was specific for lung function change predicting hypertension. Changes in blood pressure did not predict loss of lung function.[30] Although our observational findings preclude any conclusions about causal relationships, they do justify studies into the effects of respiratory interventions on the cardiovascular system.

The process of atherosclerosis starts in the young and risk factors for cardiovascular disease in childhood track into adulthood.[16, 31] Lung function is, together with smoking, one of the strongest predictors of COPD.[17, 32] Since the association of lung function and blood pressure is already present in childhood, future research to explore the association should not only focus on adults. It is important to consider prevention and early detection of chronic disease from a broader perspective.

This study provides the first evidence for a positive association of mechanical properties of the respiratory system and blood pressure in childhood and young adulthood. As cardiovascular and respiratory diseases are a major health-economic burden, improved understanding of the association between these two diseases is needed.

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Chapter 3

The association between lung function and arterial stiffness in young childhood

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Abstract

Background

In adults, precursors of cardiovascular disease are associated with lower lung function. From a preventive point of view, it is important to get a better understanding of the early life origins of these associations. We aimed to assess if an association between lung function and arterial stiffness is already present in young children.

Methods

Within the WHISTLER study 553 5-year-olds had successful spirometry (FEV1) and ultrasonography of the a. carotis communis. In 230 8-year-old children measurements were repeated. Linear regression analyses were performed with lung function as independent variable and arterial stiffness as dependent variable. Analyses were adjusted for anthropometrics and environmental factors.

Results

Higher FEV1 was associated with increased arterial stiffness (higher elastic modulus (35.1 kPa/L, 95% CI 12.6 to 57.6) and lower distensibility (-17.9 MPa-1/L, 95% CI -30.3 to -5.5)) at age 5. After adjustment for age, sex, weight and height the associations attenuated and were not statistically significant anymore. Analysis in the 8-year-olds confirmed the findings. A larger increase between lung function (ΔFEV1 5-8 years) was associated with increased arterial stiffness at age 8, which attenuated after adjustment of sex and anthropometrics.

Conclusion

Our study provides evidence that the association between impaired lung function and increased arterial stiffness as described in adulthood, is not present in childhood. In young childhood, increase in lung function is related with increased arterial stiffness. This is largely explained by anthropometry.

Introduction

Several studies have shown that reduced lung function is a risk factor for cardiovascular morbidity and mortality.[1] A meta-analysis showed that individuals in the lowest quintile of Forced Expiratory Volume in 1 second (FEV1) have a 75% higher risk for cardiovascular mortality than those in the highest quintile, even among lifetime non-smokers.[2] Not only cardiovascular morbidity and mortality are associated with lower lung function, but also precursors for cardiovascular diseases, like hypertension, arterial stiffness and intima-media thickness, independent of gender or anthropometrics.[3–11] Currently, there is no explanation for these relationships. Systemic inflammation or traditional cardiovascular risk factors such as smoking and serum cholesterol could not fully explain this relationship.[7, 8, 10]

Recent findings support the hypothesis that a common pathway exists between a decline in lung function and subsequently the development of arterial stiffness or hypertension. A study among middle-aged subjects showed that a decline in Forced Expiratory Volume (FVC) predicted incident hypertension.[11] Another study among men showed that mid-life lung function is a stronger risk factor than later-life lung function for arterial stiffness, even after taking traditional risk factors into account.[4] Suggested explanations were shared factors, like infections during childhood or postnatal growth, which could influence both lung function and arterial stiffness. Recently, we studied the association between lung function and blood pressure from neonatal till elderly age. This study provided evidence for an association between higher lung function and higher blood pressure in young individuals. This association reversed with increasing age.[12]

However, little is known about the association between arterial stiffness and lung function and about the influence of anthropometrics and environmental factors in early life. In this study, we aimed to assess if an association between lung function and common carotid artery stiffness is already present in young healthy children.

Methods

Setting and participants

This study is part of the Wheezing Illnesses Study Leidsche Rijn (WHISTLER), which is an ongoing birth cohort study initiated in 2001. The design and rationale of the study is described elsewhere.[13] Healthy infants are invited to participate before the age of 2 months. Exclusion criteria are gestational age <36 weeks, neonatal respiratory disease and congenital abnormalities. Infants in the Netherlands regularly visit Child Health Care Centres for weight and length measurements. Parents were asked to report these measurements of weight and height in a monthly questionnaire during the first year. This study was approved by the Medical Ethics Committee of the University Medical Centre Utrecht and conducted according to the Declaration of Helsinki. Written informed consent was obtained from all parents of the participants.

Measurements

In November 2007, the study was extended with measurements of the carotid artery. At the age of 5 and 8 years the participants were re-invited to visit the outpatient clinic. At these two visits the same measurements were performed according to the same protocol. A detailed overview of included participants at these ages is presented in figure 1. In total, 184 children had a successful spirometry at the age of 5 and 8 years and a vascular measurement at the age of 8 years. Prior to each visit a health questionnaire containing information about pre- and postnatal risk factors and about health status of both the participant and parents was completed by the parents.

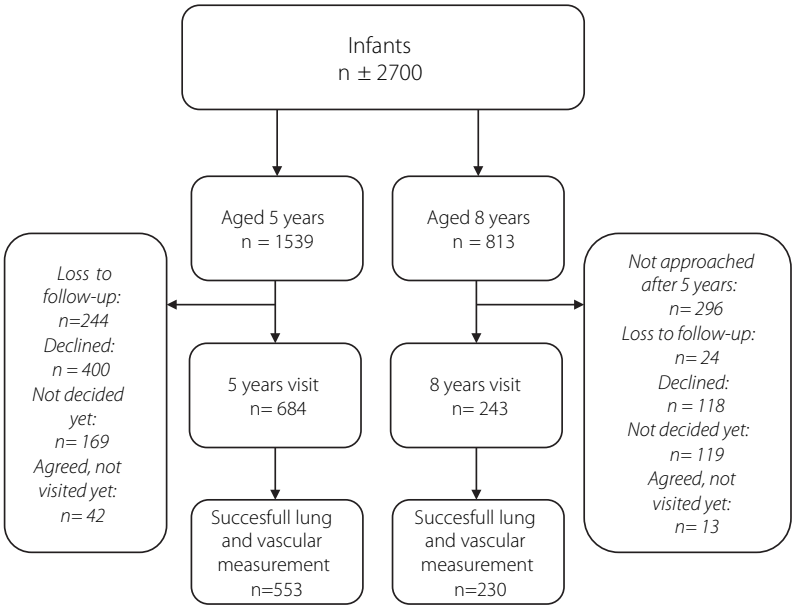

Figure 1. *Overview of the WHISTLER study population (July 2012)*

Lung function

Lung function was measured using a heated Lilly head pneumotachometer system (Viasys Healthcare, Hochberg, Germany) conform the latest American Thoracic Society (ATS)/ European Respiratory Society (ERS) statement for lung function measurements in preschoolers.[14] Measurements were corrected for body temperature, pressure and saturation. At least two reproducible flow-volume curves were obtained. The largest forced expiratory volume in 1 second (FEV1) was selected.

Vascular measurements

Measurement of the vascular system is already extensively described elsewhere.[15] Briefly, vascular conditions of the right common carotid artery were studied by using high-resolution echo-tracking

technology (Art.lab, Esaote, Italy) using a 128 radiofrequency line multiarray, with a L10-5 40 mm linear array transducer. Raw radiofrequency data were analysed online and 6-second cineloops were stored without compression (120 Mbytes) for offline analysis. Children were measured in a supine position after at least 10 minutes of rest. All major mechanical parameters for a 4-cm arterial segment were measured. These parameters include diastolic diameter, change in diameter from systole to diastole (distension), distension as a function of time and carotid intima-media thickness (cIMT). Averages of cIMT, lumen diameter, and distension were used to assess the elastic properties of the artery as a hollow structure through cross-sectional distensibility, and of the arterial wall through the elastic modulus. Both a lower distensibility and a higher elastic modulus indicate a stiffer artery. The formulas and units are described in the supplement. Measurements were repeated a maximum of four times. All measurements were performed by trained personnel. Reproducibility was evaluated based on measurements by one observer in 10 subjects on two different occasions. Coefficients of variation for distension and lumen diameter were 7.1, 4.4 and 2.4%, respectively.[16]

During ultrasonography, blood pressure was measured twice at the brachial artery using a semiautomatic oscillometric device (DINAMAP; Criticon, Tampa, FL). The average of both measurements was used for analyses.

Anthropometric measurements

Weight was measured in light clothing using a standard electronic scale and height without wearing shoes using a stadiometer. Standing with the feet slightly apart waist circumference was measured twice at the level midway the lowest rib border and the iliac crest.

Intra-abdominal and subcutaneous adipose tissue were measured using ultrasound according to a previously described procedure with a Picus Pro system (Esaote, Italy), using a CA 421 convex transducer.[15] For intra-abdominal adipose tissue, the distances between the posterior edge of the abdominal muscles and the lumbar spine were measured using electronic callipers. Distances were measured from three different angles: medial, left and right lateral, with the transducer placed longitudinally on a straight line drawn between the left and right midpoint of the lower rib and iliac crest. Measurements were performed at the end of a quiet expiration. The average distance was calculated from the three angles. Placing the probe transversely at the level of the umbilicus, sonographic subcutaneous adipose tissue was measured with electronic callipers from the external face of the rectus abdominis muscle (linea alba) to just below the skin. The measurement was repeated three times and the average was used for analysis. For all measurements, minimal pressure was applied to eliminate manual compression of tissue. Intraclass correlation coefficients (ICC) based on measurements by one observer in 10 subjects for intra-abdominal fat and, respectively 11 subjects for subcutaneous fat on 2 different occasions were 0.67 and 0.96, respectively. ICCs for subcutaneous fat on the three measurements per child on the same occasion were 0.94, 0.94, and 0.97 for the three observers.[17]

History of infections and allergies

General practitioner (GP) diagnosed allergies and infections using the International Classification of Primary Care (ICPC) codes were obtained from the GPs electronic files (Medicom, PharmaPartners, the Netherlands) from birth until the date of visit (at age 5 and 8 years). An ever diagnosed allergy was defined according to the following ICPC codes: allergic reaction (A12), allergic conjunctivitis (F71), asthma (R96), allergic rhinitis (R97), constitutional eczema (S87) and urticaria (S98).

GP diagnosed infections were defined according to the infectious diseases subgroup of ICPC component seven. Per child the number of GP diagnosed infections from birth until the date of visit was summed, as measure of cumulative infection exposure. We developed 4 categories. The first group experienced 0-2 infections till the age at visit, the second group 3-5 infections, the third group 6-9 and the last group >10 infections till the age at visit.

Data analysis

Central estimators and variance measures to describe general characteristics were calculated. All variables were checked for normality of distribution. Linear regression analyses were performed to study the association between lung function (FEV1, Forced Volume Capacity (FVC) and Peak Expiratory Flow (PEF)) as independent variable and characteristics of the vascular system (distensibility and elastic modulus) at the age of 5 and 8 years as separate dependent variables.

In multivariable linear regression analyses we assessed the influence of anthropometrics and environmental factors in separate models. First, we developed four models. The first univariable model contained only our determinant of interest. In the second model sex, age, height and weight were added. The third model included early environmental factors (during pregnancy and the first year of life). These factors were growth in the first three months of life and tobacco smoke exposure during pregnancy. Growth in the first months of life has shown to be an important predictor of lung function and arterial stiffness.[18, 19] For the adjustment of postnatal growth we used the weight gain rate of at least two measurements available in the first 3 months adjusted for the length gain rate. This method was previously described.[18] Tobacco smoke exposure was categorized as smoke exposure of mother during pregnancy and current smoke exposure due to smoking of one of the parents. The fourth model contained factors of which exposure could accumulate with age. These factors include tobacco smoke exposure, experienced infections and ever diagnosed allergies by the general practitioner till the age of visit.

Second, we assessed the influence of factors associated with body weight, an important determinant of both lung function and properties of the vascular system.[15] We studied the influence of body weight, waist circumference, subcutaneous and intra-abdominal adipose tissue by a multivariable regression analysis in the same way as described above.

Finally, as a previous study had shown that lung function in mid-life is a stronger risk factor than in later life for arterial stiffness[4], we assessed the association between lung function at age 5 years and arterial stiffness at age 8 years. Furthermore, we assessed if the absolute rate of change between lung function at age 8 and age 5 years is associated with arterial stiffness at age 8 years.

Table 1. *Baseline characteristics of children at age 5- and 8-years*

Patient Characteristics		Visit at age 5 years			Visit at age 8 years		
		N	Mean	SD	N	Mean	SD
Sex (n, %)	Female	290	52.4		134	58.3	
	Male	263	47.6		96	41.7	
Age (years)		553	5.4	0.26	230	8.1	0.42
Height (cm)		553	114.9	4.75	230	132.3	5.97
Weight (kg)		553	20.2	2.79	230	28.0	4.79
Waist circumference (cm)		547	56.0	3.2	228	62.8	4.56
Intra-abdominal adipose tissue (mm)		517	36.3	6.4	229	37.7	7.27
Subcutaneous adipose tissue (mm)		545	6.8	3.2	228	8.52	5.15
Mean weight gain (g/day)		467	28.4	5.8	192	28.6	5.76
Mean length gain (mm/day)		466	1.1	0.1	193	1.1	0.1
Smoking of one of the parents between birth and the visit (n, %)	No	327	59.1		142	61.7	
	Yes	140	25.3		48	20.9	
	Missing	86	15.6		40	17.4	
Maternal smoke exposure during pregnancy (n, %)	No	411	74.3		155	67.4	
	Yes	127	23.0		72	31.3	
	Missing	13	2.7		3	1.3	
No. of experienced infections till visit (n, %)	0 - 2	106	19.2		32	13.9	
	3 - 5	119	21.5		50	21.7	
	6 - 9	107	19.3		53	23.0	
	> 10	134	24.2		59	25.7	
	Missing	87	15.8		36	15.7	
Ever diagnosed allergy by general practitioner (n, %)	No	290	52.4		108	47.0	
	Yes	176	31.8		86	37.4	
	Missing	87	15.8		36	15.6	
Lung function							
FEV1 (L)		553	1.28	0.19	230	1.78	0.26
FVC (L)		553	1.36	0.22	230	1.96	0.29
PEF ($L \cdot s^{-1}$)		553	2.81	0.54	230	3.98	0.69
Vascular measurement							
Systolic blood pressure (mm Hg)		535	103	7.4	230	106	7.3
Diastolic blood pressure (mm Hg)		535	53	6.2	230	54	6.0
Heart rate (rate/min)		534	86	8.8	230	72	5.6
Carotid distensibility (MPa^{-1})		475	95.9	26.3	230	85.7	21.7
Carotid Elastic Modulus (kPa)		462	158.7	47.4	230	189.0	58.3

Values are counts (N), means and standard deviations (SD) unless otherwise indicated.

Missing data on determinants were imputed using the automatic multiple imputation method of SPSS. All variables used in the later statistical analysis and the outcome were used as predictor.[20] The analysis was performed in 10 imputed data sets and the pooled results are expressed as linear regression coefficients and 95% confidence intervals. Confidence intervals not including zero and p-values <0.05 were considered statistically significant. All analyses were performed using IBM SPSS Statistics, version 20.0 (Armonk, New York, USA).

Results

Table 1 describes the baseline characteristics of participants at the age of 5- and 8- years with a successful lung function and vascular measurement.

Table 2. *Association between lung function (FEV1) and arterial stiffness.*

	Age 5 years		Age 8 years	
Model	**B***	**95% CI**	**B***	**95% CI**
Distensibility (MPa^{-1})				
1	**-17.89**	**-30.27, -5.50**	**-17.75**	**-28.26, -7.25**
2	-7.67	-23.34, 8.00	-4.04	-17.56, 9.49
3	**-17.48**	**-29.94, -5.01**	**-16.71**	**-27.26, -6.17**
4	**-17.76**	**-30.21, -5.30**	**-18.35**	**-28.94, -7.76**
Elastic Modulus (kPa)				
1	**35.08**	**12.60, 57.55**	**47.17**	**18.84, 75.50**
2	19.49	-8.77, 47.75	7.89	-28.46, 44.24
3	**35.32**	**12.69, 57.94**	**46.44**	**17.87, 75.02**
4	**34.43**	**11.82, 57.05**	**47.29**	**18.85, 75.74**

**The numbers are linear regression coefficients representing difference in vascular outcomes per unit increase in FEV1 (L) based on the imputed datasets.*

Model 1: no adjustments
Model 2: age, sex, height, weight
Model 3: smoke exposure mother during pregnancy, 3 months postnatal growth
Model 4: current smoking of one of the parents, infections and allergy diagnosed by the general practitioner

Table 2 shows the linear regression coefficients for the association of lung function and vascular parameters in the different models. In the univariable model there was a relation between lower FEV1 and increased arterial stiffness (higher distensibility and lower elastic modulus). After adjustment for age, sex, weight and height there was no statistically significant association anymore (model 2). Adjustment for early environmental factors and adjustment for smoking and experienced infections or allergies in model 3 and 4 did not affect the association. The results at the age of 8 years were similar to those at 5 years. The models with Forced Vital Capacity (FVC) as dependent variable showed similar results in the 5-year-old children. The linear regression coefficients had a similar trend in the 8-year-olds, but did not reach statistical significance (data not shown).

In table 3 we assessed the influence of weight associated factors on the associations between lung function and the vasculature. After adjustment for height and sex in the 5-year-olds, lung function was statistically significantly related to elastic modulus only. Weight, intra-abdominal adipose tissue and waist circumference further attenuated the relations for lung function. Subcutaneous adipose tissue barely changed the association. At the age of 8 years we found comparable effects of adjustments, but changes in the regression coefficients in this age group were much smaller than those found at age 5 years.

Table 3. *Association between lung function (FEV1) and arterial stiffness after adjustment for weight associated factors.*

	Age 5 years		Age 8 years	
Model	**B***	**95% CI**	**B***	**95% CI**
Distensibility (MPa^{-1})				
1	-11.35	-26.75, 4.05	-5.84	-19.07, 7.39
2	-7.76	-23.4, 7.89	-3.95	-17.23, 9.34
3	-8.90	-24.07, 6.27	-5.01	-17.71, 7.68
4	-10.89	-26.29, 4.51	-6.13	-12.91, 0.64
5	-8.20	-23.52, 7.12	-4.93	-18.3, 8.45
Elastic Modulus (kPa)				
1	**29.13**	**1.24, 57.03**	13.77	-21.89, 49.44
2	20.18	-8.01, 48.38	8.01	-27.71, 43.74
3	24.81	-2.73, 52.36	11.96	-22.97, 46.88
4	**27.98**	**0.01, 55.95**	13.48	-22.34, 49.30
5	21.91	-5.52, 49.35	12.86	-22.04, 47.75

**The numbers are linear regression coefficients representing difference in vascular outcomes per unit increase in FEV1 (L) based on the imputed datasets.*

Model 1: Sex, height
Model 2: Sex, height, weight
Model 3: Sex, height, intra-abdominal adipose tissue
Model 4: Sex, height, subcutaneous adipose tissue
Model 5: Sex, height, waist circumference

Table 4 shows the results of the association between lung function at the age of 5 years and arterial stiffness at the age of 8 years. Higher lung function at age 5 was associated with higher elastic modulus (51.93 kPa/L, 95% CI 8.22- 95.65) and marginally associated with lower distensibility at age 8 (-16.74 MPa-1/L, 95% CI -33.56 to 0.09), but after adjustment for anthropometrics the direction of the regression coefficients reversed.

Table 4. *Lung function (FEV1) at the age of 5 years and absolute change in FEV1 between 5 and 8 years as a predictor for arterial stiffness at the age of 8 years.*

	FEV1 (L) at age 5 years		Δ FEV1 (L)	
Model	**B***	**95% CI**	**B***	**95% CI**
Distensibility (MPa^{-1})				
1	-16.74	-33.56, 0.09	**-25.42**	**-42.42, -8.42**
2	8.04	-12.03, 28.11	-14.65	-32.04, 2.73
3	-15.39	-32.34, 1.55	**-26.14**	**-43.26, -9.01**
4	**-19.85**	**-36.74, -2.96**	**-23.95**	**-41.30, -6.61**
Elastic Modulus(kPa)				
1	**51.93**	**8.22, 95.65**	**63.96**	**19.51, 108.40**
2	-14.21	-65.70, 37.29	33.30	-11.34, 77.93
3	**50.60**	**6.47, 94.73**	**67.36**	**22.54, 112.19**
4	**59.65**	**15.91, 103.39**	**59.91**	**14.62, 105.20**

**The numbers are linear regression coefficients representing difference in vascular outcomes per unit increase in FEV1 (L) based on the imputed datasets.*

Model 1: no adjustments
Model 2: sex, height, weight
Model 3: smoke exposure mother during pregnancy, 3 months postnatal growth
Model 4: current smoking of one of the parents, infections and allergy diagnosed by the general practitioner

A larger increase between lung function (FEV1) from age 5 to 8 years (Δ FEV1 (L)) was associated with increased arterial stiffness (linear regression coefficients -25.42 MPa-1/L, 95% CI -42.42 to -8.42 and 63.96 kPa/L, 95% CI 19.51 to 108.4 for distensibility and elastic modulus, respectively). After adjustment for sex, weight and height at 8 years the associations were not statistically significant anymore. Adjustment for environmental factors alone did not affect the association.

Discussion

Our study provides evidence that in childhood higher lung function is associated with higher arterial stiffness. This association was largely explained by anthropometry, while environmental factors alone did not affect the association.

To interpret the results of this study, some issues need to be considered. Some of our data is self-reported, and possibly imprecise. For instance, parental smoking was self-reported, although our data do seem to reflect real smoking habits.[21, 22] However, certainly not all measured (developmental) factors were self-reported. The visits to the general practitioners were directly extracted from their electronic medical records, clearly more objective than self-reported diagnosis. Information about postnatal growth was prospectively gathered. We have also adjusted for the number of infections diagnosed by the general practitioner, as a surrogate of inflammation. During an infection, inflammatory mediators will be released.[23, 24] Children who experience more infections during childhood will probably be more

exposed to inflammatory biomarkers. Inflammatory mediators are associated with a decrease in lung function[25, 26] and increase in vascular stiffness[27]. Although we cannot exclude the possibility of residual confounding, we do believe to have maximally addressed this issue given the measurements that we have available.

Flow-volume measurements are often difficult in young children, because they may lack coordination and cooperation. In our study we chose to look at FEV1 as the primary measure of lung function, because as shown by a previous study, FEV1 has the highest reproducibility compared to FVC in young children.[28] Furthermore, PEF is effort-dependent, so maximum subject cooperation is essential, while FEV1 is less effort dependent.[14]

We feel that our study has several strengths. To our best knowledge, our vascular measurements at the age of 5 years are the youngest reported in a large group of healthy children. Given the complexity of measurements, our sample size is substantial. Moreover, our study adds to insights on the association between individual change in lung function and arterial wall characteristics.

The directions of our findings are consistent with other studies. In a cross-sectional study performed in individuals aged ≥ 20 years, the relationship between pulse pressure, an indirect measurement of arterial stiffness, and lung function was examined. Higher FEV1 was associated with lower pulse pressure among those aged ≥ 40 years, but below 40 years an opposite direction was found, although not statistically significant anymore after adjustment for confounders.[8] Recently, we performed a similar study to study the relationship between blood pressure (including pulse pressure) and lung function from neonatal till elderly age. This study showed that in contrast to findings in elderly, in young individuals higher lung function is associated with higher blood pressure. This association reversed with increasing age.[12] Given the positive relation between blood pressure and thicker and stiffer arteries at this age,[29] our previous findings with blood pressure would agree with our present findings. However, findings opposite to our current results have been presented as well. A study among 249 8-year-old children showed that lower lung volumes are associated with increased arterial stiffness, measured by the augmentation index.[30] The different findings from that study and ours are most likely due to differences in participants and methodology. The participants of that study were at high familial risk for development of asthma, while asthmatic children have a higher intima-media thickness than healthy controls. Selection on asthma proneness could have induced both predominance of lower lung volumes and stiffer arteries. In that study, there was only adjustment for height, while we found quite strong effects of weight adjustment as well.

A longitudinal study in adult men has shown that mid-life lung function is a stronger risk factor than later-life lung function for arterial stiffness, also after accounting for traditional cardiovascular risk factors. The authors suggested that this finding may be explained by factors like postnatal growth and infections during childhood.[4] In our study, we had information of these factors, including information from general practitioners. We did adjust for many potentially important environmental factors, but found no material influence. Anthropometry, on the other hand, did have an influence. At the age of 5 years, there was a positive association between FEV1 and elastic modulus, also after adjustment for sex and height.

However, after adjustment for weight, waist circumference or intra-abdominal adipose tissue there was no association between lung function and arterial stiffness, while adjustment for subcutaneous adipose tissue showed only a slightly attenuated association. This would be consistent with previous reports that only visceral (intra-abdominal) and not subcutaneous adipose tissue is associated with increased arterial stiffness in children.[15] Fat mass in non-obese children, calculated from triceps skinfold thickness and arm circumference, has been shown associated with a decrease of spirometric parameters.[31] Another study in children relating anthropometric measurements with lung function showed opposite results.[32] In this study a higher waist circumference was associated with a more favourable lung function, which is consistent with our findings.[32] But as far as we know, the relationships between lung function and various specific deposits of adipose tissue have not yet been studied in healthy children. In adult patients with asthma an increase in both subcutaneous and visceral adipose tissue are associated with lower lung function.[33] In healthy adults conflicting results about the influence of subcutaneous or visceral adipose tissue on lung function have been presented.

Currently, it is unknown if properties of the vascular system in the young have the same pathophysiological implications as in adults. A longitudinal study with a long-term follow-up is necessary to study the correlation between arterial stiffness in childhood and cardiovascular morbidity and mortality in adulthood. However, as described above, an unfavourable cardiovascular risk profile in young adulthood and childhood is already associated with an increase in intima-media thickness and arterial stiffness. Probably, the association between lower lung function and higher arterial stiffness described in adulthood develops later in life. The biological pathway linking respiratory function and arterial stiffness is still unknown, but as shown by our study in childhood this is largely explained by anthropometry and not environmental factors. Based on previous literature,[2] it can be speculated that the reversal of the association might be due to prolonged exposure to pulmonary and cardiovascular risk factors. A second explanation might be tissue stiffening (aging) of both the pulmonary and vascular system over time. Third, the changes in pulmonary and vascular system over time might be directly causally related. Longer follow-up is needed to give a better understanding of this reversal.

Many studies have shown that lung function is risk factor for cardiovascular disease. It is known that early life adverse exposures could lead to permanent metabolic or structural changes, which in later life result in cardiovascular disease.[34, 35] From a preventive point of view, it is important to get a better understanding of the early life origins of this association. It is probably too soon for practical purposes but there is a need for studies on early origins of associations that are becoming quite clear in later life, e.g. lung and heart disease. This is the first study exploring the association between lung function and arterial stiffness in a large group of healthy children, measured at two different ages.

In conclusion, our study provides evidence that the association between impaired lung function and increased arterial stiffness as described in adulthood, is not present in childhood, but probably emerges later in life. The univariable association between higher lung function and increased arterial stiffness is lost after adjusting for age, sex, weight and height.

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Supplement Equations of vascular parameters

Δd (change in carotid diameter)	$d_{systolic} - d$
Δp (carotid pulse pressure)	CF * Δd
CF (conversion factor)	$(MAP - DBP)/(d_{mean} - d)$
MAP (mean arterial pressure)	DBP + (SBP − DBP)/3
Δ A (change in arterial cross-sectional area)	$\pi/4 * [(d + \Delta d)^2 - d^2]$
DC	$(\Delta A / A) / \Delta p = (2 \Delta d * d + \Delta d^2) / (\Delta p * d^2)$
EM	(d/IMT) / DC
$d_{systolic}$	mean end-systolic lumen-IMT diameter (mm)
d	mean end-diastolic lumen-IMT diameter (mm)
A	arterial cross-sectional area (mm^2)
DC	distensibility coefficient (1/MPa)
EM	elasticity, Young's modulus (kPa)
CIMT	Carotid intima-media thickness, end-diastolic (mm)
SBP	systolic blood pressure in brachial artery (mmHg)
DBP	diastolic blood pressure in brachial artery (mmHg)
MAP	mean arterial pressure in brachial artery (mmHg)

Chapter 4

The cardiovascular system in children with cystic fibrosis

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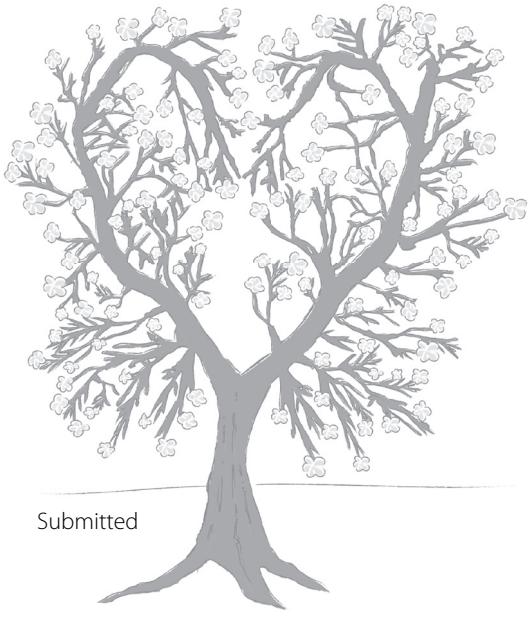

Submitted

Abstract

Background

As life expectancy is increasing in patients with cystic fibrosis, it is important to pay attention to extra-pulmonary comorbidities. Several studies have shown signs of myocardial dysfunction in adult patients, but little is known about onset and development of these changes over time. In this study cardiac function in children with cystic fibrosis is compared with that of healthy children.

Methods

Children, aged 3-12 years, with cystic fibrosis were recruited from the Wilhelmina Children's hospital and age-matched healthy children were selected from the WHISTLER study, a population-based cohort study. Measurements of lung function, arterial stiffness, and echocardiography (conventional measures and myocardial LV and RV deformation imaging) were performed.

Results

There were no differences in anthropometrics, lung function and blood pressure between the two groups. The cystic fibrosis children had a higher arterial stiffness compared to the healthy children (pulse wave velocity respectively 5.76 ± 0.57 m/s versus 5.43 ± 0.61 m/s, p-value 0.05). Echocardiography showed a reduced systolic function of the right ventricle compared to the healthy children. Myocardial strain assessment showed a trend towards a reduced strain and strain rate of both the right and left ventricle in children with cystic fibrosis compared to healthy children. Global strain of the left ventricle in cystic fibrosis children was -21.56±2.66% versus -22.70±1.61% (p-value 0.05) in the healthy children and for the right ventricle respectively -29.46±5.32 versus -32.00±4.58% (p-value 0.07).

Conclusion

Already at a very young age, children with cystic fibrosis in a good clinical condition show an increased arterial stiffness and signs of diminished both right and left ventricular function.

Introduction

Cystic fibrosis (CF) is a genetic disorder, which mainly affects the lungs. Recent improvements in treatment and screening options will increase the life expectancy of adult patients with cystic fibrosis. Therefore, it has become important to pay attention to extra-pulmonary comorbidities that affect the length and quality of life in the adult stage of the disease. Currently, cardiovascular involvement, like pulmonary hypertension or heart failure, becomes manifest in a late stage of the disease.[1] Several studies have shown that subclinical signs of dysfunction of the heart is already present in an earlier stage of the disease.[2–5] Most studies focussed on the right ventricle,[2–4] but subclinical dysfunction of the left ventricle in adult patients with cystic fibrosis is also described.[5] In addition to changes in cardiac function, increased arterial stiffness has been described to be present in adult patients with CF as well.[6, 7] Little is known about onset and course over time of these cardiovascular changes. Whether cardiovascular changes occur as a result of problems inherent to the disease, like pulmonary changes, chronic infections or diabetes mellitus, or whether the CF transmembrane regulator (CFTR) dysfunction is directly related to impairment of cardiac function remains unknown.

The aim of this study was to assess the cardiovascular system, including both right and left ventricular function and arterial stiffness before the above-mentioned problems associated with CF have played a substantial role. Therefore we studied cardiovascular parameters in young children with CF with healthy peers as controls.

Material and methods

Setting and participants

We selected children, aged 3-12 years with a confirmed diagnosis of CF, who were in a stable respiratory condition at the time of visit. These children were recruited from the Wilhelmina Children's Hospital from January till September 2013. During the same period, age-matched healthy children were selected from the Wheezing Illnesses Study Leidsche Rijn (WHISTLER), a population-based birth cohort study. Study design and rationale of WHISTLER were described in detail elsewhere.[8] Exclusion criterion for the current study was any congenital heart disease. The paediatric medical ethics committee of the University Medical Center Utrecht, the Netherlands, approved the study. Written informed consent was obtained from the parents.

Visit

All children were invited to visit our outpatient clinic for respiratory and cardiovascular measurements. Prior to this visit a health questionnaire containing information about risk factors and health status of both the participant and parents was completed by the parents. During the visit, weight of the children was measured in light clothing using a standard electronic scale and height without wearing shoes using a stadiometer.

Lung function

Interrupter resistance was measured using MicroRint (MicroMedical, VIASYS Healthcare, Kent, UK) according to previous described methods.[9]

Spirometry was performed in children ≥5 years using the ZAN spirometer (ZAN 100, nSpire Health Inc., Germany). Measurements were performed according to the latest American Thoracic Society (ATS)/ European Respiratory Society (ERS) statement for lung function measurements in preschoolers.[9] Forced Expiratory Volume in 1 second (FEV1), Forced Vital Capacity (FVC) and and Peak Expiratory Flow (PEF) were expressed as percent predicted for the patient's age, height and sex according to prediction equations previously published.[10]

Blood pressure and arterial stiffness

Blood pressure and aortic pulse wave velocity, a measure of arterial stiffness, were measured at rest in a calm and supine position using the Arteriograph (Tensiomed, Budapest, Hungary) at the end of the echocardiographic examination of ±30 minutes. The Arteriograph is a non-invasive oscillometric device of which method and validation have been described previously.[11–13] A higher pulse wave velocity indicates a stiffer artery.

Echocardiography

Transthoracic echocardiographic imaging was performed using a ViVid 7 ultrasound machine (GE Healthcare, Milwaukee, WI, Wauwatosa, U.S.A.) with a 6S, 2.7-8.0 MHz transducer with continuous electrocardiographic monitoring. All measurements were performed at rest without using sedation, with the children lying in a supine left lateral position. The acquisition of the echocardiograms was done by one investigator (JE) who had had a dedicated training in echocardiography prior to the study. All measurements were performed using the recommendations of the paediatric council of the American Society of Echocardiography.[14, 15] All echo analyses were done by two investigators (AT for all deformation imaging measurements and MV for all other measurements) who were blinded to the diagnosis of the children. Mean heart rate was calculated from at least three randomly selected separate images of the echocardiogram.

Longitudinal strain analysis was performed by one experienced investigator (AT) in this field.[16, 17] We previously described in detail our methods for image acquisition and post processing for speckle tracking.[17] In brief, we acquired wide angle, real time two-dimensional ultrasound data from the septal and lateral wall in the apical 4-chamber view at a frame rate of (49 to 99 Hz). Additionally, ultrasound data from the right ventricular free wall was recorded in the apical view. The image sector width was set as narrow as possible and depth was adjusted to exclude the atrium from the image view (frame rate of 49 to 111 Hz).

B-mode images of one cardiac cycle of the right ventricular free wall and the left ventricle were used to extract 2D strain and strain-rate curves. Commercially available software (EchoPAC PC 2D-strain, GE Vingmed Ultrasound, Milwaukee, Wis) using a two step tracking algorithm was used. A region of interest (ROI) was manually traced along the endocardial border from base to apex at the end of systole and the ROI-width was set to match the wall thickness. The tracked ROI was visually checked and adjusted

if necessary. The ROI was divided into three segments (basal, mid and apical). The calculated values for strain and strain-rate were averages over entire myocardial segments. Only inappropriate tracking and drop out from the image plane resulted in exclusion of the myocardial segment from analysis. The starting point of the strain-curve was placed at the onset of the QRS complex on the ECG. Drift compensation was applied and temporal and spatial smoothing was set to the default setting of the application. End systole was defined at the time of the aortic and pulmonic valve closure in the left and right ventricular deformation graphs, respectively.
Of each segment, peak systolic strain (%) and peak systolic strain-rate (1/s) were defined. Additionally, global peak systolic strain and global peak systolic strain-rate was calculated as an average of both the right ventricular free wall (3 segments) and of the left ventricle (6 segments).

Statistical analysis

Central estimators and variance measures to describe general characteristics were calculated. All variables were checked for normality of distribution. Differences in characteristics of children with CF versus healthy control children were tested using independent samples T-test for continuous variables or χ^2 for categorical variables. Using linear regression analyses we further explored the association, with CF as independent variable and global strain (%) of left and right ventricle as dependent variable. In multivariable regression models we adjusted for weight, height and heart rate. Statistical significance was considered reached at p-value <0.05. All analyses were performed with SPSS version 20.0 for Windows (IBM, Armonk, New York, USA).

Results

We studied 33 patients with CF and 33 age-matched healthy children. In total, 31 of the 33 CF children had mutations with only minimal CFTR function (class I, II or III), of which 26 children had a homozygous ΔF508 mutation. The remaining two children had a class V mutation, which is associated with reduced expression of CFTR and a mild phenotype.[18] One of the children in the CF group had diabetes mellitus.
Table 1 shows the baseline characteristics of the children with CF and the healthy children. The mean age of the children was 8.5 years. There were no significant differences in baseline characteristics between the CF and the healthy children. In total, 25 children per group were aged ≥5 years and were able to perform a spirometry. Both the healthy and CF children had a mean lung function above 100% predicted. There was a trend of a lower FEV1 in the CF children compared to the healthy children, but this was not significant (107.3 ± 16.1% respectively 112.7 ± 15.9%, p-value 0.25). The lowest FEV1 was 74% of a CF patient.

The results of the comparison of blood pressure and arterial stiffness are shown in table 2. The CF children had a higher pulse wave velocity compared to the healthy children (respectively 5.76 ± 0.57 m/s versus 5.43 ± 0.61 m/s, p-value 0.05). No differences in systemic blood pressure were observed between the two groups.

Table 1. *Baseline characteristics*

		Healthy children (n=33)		Cystic Fibrosis (n=33)		
		Mean	**SD**	**Mean**	**SD**	**p-value**
Sex (%, n)	Male	46.7	14	53.3	16	0.62
	Female	52.8	19	47.2	17	
Age (years)		8.5	2.7	8.5	2.7	0.97
Height (cm)		132.2	17	132.2	17.6	0.99
Weight (kg)		29.4	9.1	28.4	7.8	0.63
Thoracic circumference (cm)		63.7	7.1	64.1	6.6	0.83
Waist circumference (cm)		60.2	7.3	61.5	5.7	0.44
Hip circumference (cm)		61.6	7.6	60.4	6.5	0.49
Lung function						
Interrupter resistance (kPa/L)		0.66	0.16	0.68	0.24	0.72
FEV1 (%)		112.7	15.9	107.3	16.1	0.25
FVC (%)		113.1	16.7	112.7	16.8	0.92
PEF (%)		134.6	22.4	132.4	16.1	0.69

FEV1: Forced Expiratory Volume in 1 second, FVC: Forced Vital Capacity, PEF: Peak Expiratory Flow.
Values are means and standard deviations (SD) unless otherwise indicated.

Table 2. *Arterial stiffness*

	Healthy children (n=33)		Cystic Fibrosis (n=33)		
	Mean	**SD**	**Mean**	**SD**	**p-value**
Arteriograph					
PWVao	5.43	0.61	5.76	0.57	0.05
Systolic blood pressure (mm Hg)	100.2	10.4	101.2	8.2	0.69
Diastolic blood pressure (mm Hg)	53.2	7.5	53.6	6.6	0.84
Mean arterial pressure (mm Hg)	68.9	8.0	69.5	6.6	0.78

PWVao: aortic Pulse Wave Velocity.

Table 3 shows the differences between echocardiograph parameters measured in the CF and healthy children using conventional methods and Tissue Doppler Imaging. Heart rate in patients with CF was slightly higher compared to the healthy children (mean heart rate respectively 82.2 ± 15.4 and 76.6 ± 11.6 per minute, p-value 0.10), however this difference was not significant. There were no differences in left and right ventricular dimensions between the two groups. Global left ventricular (LV) function measured by LV fractional shortening and velocity of the S' wave measured with Tissue Doppler Imaging, showed no differences between the cystic fibrosis and the healthy group. Systolic function of the right ventricle, measured by Tricuspid Annular Plane Systolic Excursion (TAPSE) and Tissue Doppler Imaging, was significantly lower in the children with cystic fibrosis (respectively CF versus the healthy children for

TAPSE 18.74 ± 2.69 mm versus 20.35 ± 3.09 mm, p-value 0.03 and TDI systolic velocity 11.91 ± 1.62 cm/s versus 12.93 ± 1.85 cm/s, p-value 0.02).

Table 3. *Comparison of conventional and Tissue Doppler Imaging derived parameters of the heart between the CF and healthy control group*

	Healthy children (n=33)		Cystic Fibrosis (n=33)		
	Mean	**SD**	**Mean**	**SD**	**p-value**
Heart rate (per min)	76.6	11.6	82.2	15.4	0.10
Dimensions					
IVSd (cm)	0.59	0.10	0.62	0.10	0.36
LVEDD (cm)	4.03	0.43	3.93	0.41	0.33
LVPWd (cm)	0.61	0.08	0.62	0.12	0.63
LVESD (cm)	2.79	0.36	2.67	0.37	0.17
RV length (cm)	6.05	0.79	5.96	0.77	0.64
RV diameter (cm)	3.54	0.63	3.47	0.88	0.74
Systolic function					
Left ventricle					
Fractional Shortening (%)	30.68	5.45	31.98	6.17	0.37
TDI s' wave (cm/s)	10.18	1.46	10.37	1.76	0.64
Right ventricle					
TAPSE (mm)	20.35	3.09	18.74	2.69	0.03
TDI s' wave (cm/s)	12.93	1.85	11.91	1.62	0.02
Septum					
TDI s' wave (cm/s)	7.56	0.72	7.36	0.67	0.25
Diastolic function					
Left venticle					
MV E (m/s)	0.99	0.19	0.99	0.58	0.99
MV A (m/s)	0.48	0.08	0.48	0.14	0.85
E/A	2.11	0.53	2.33	1.92	0.53
E/e'	5.06	1.09	5.40	3.15	0.56
Right ventricle					
TV E (m/s)	0.77	0.10	0.69	0.10	<0.01
TV A (m/s)	0.31	0.08	0.31	0.09	0.95
E/A	2.58	0.69	2.39	0.71	0.29
E/e'	5.10	0.98	5.21	1.65	0.74
TDI e' wave (cm/s)	15.37	2.33	14.12	2.98	0.06
TDI a' wave (cm/s)	9.16	9.83	7.86	2.27	0.46

IVSd: interventricular septum in diastole, LVEDD: left ventricular end-diastolic dimension, LVPWd: left ventricular posterior wall in diastole, LVESD: left ventricular end-systolic dimension, RV length: right ventricular length, RV diameter: right ventricular diameter, TDI s' wave: Systolic velocity measured using Tissue Doppler Imaging, TAPSE: tricuspid annular plane systolic excursion. MV E: Mitral Valve, TDI e' wave: Early diastolic velocity measured using Tissue Doppler Imaging, TDI a' wave: Late diastolic velocity measured using Tissue Doppler Imaging.

Table 4. *Comparison of myocardial strain assessment between the CF and healthy peers group*

	Healthy children (n=33)		Cystic Fibrosis (n=33)		
	Mean	**SD**	**Mean**	**SD**	**p-value**
Left ventricle					
Global strain (%)	-22.70	1.61	-21.56	2.66	0.05
Global strain rate (per sec)					
SRe	-1.95	0.44	-1.84	0.46	0.38
SRa	2.58	0.59	2.32	0.57	0.13
SRs	1.17	0.53	1.20	0.59	0.90
Systolic strain (%)					
Base	-20.58	2.82	-18.68	3.35	0.03
Mid	-24.95	3.21	-23.77	4.24	0.23
Apical	-25.46	4.10	-24.31	5.44	0.36
Strain rate					
Base	-1.29	0.37	-1.17	0.30	0.22
Mid	-1.44	0.30	-1.35	0.25	0.22
Apical	-1.63	0.39	-1.61	0.30	0.86
Right ventricle					
Global strain (%)	-32.00	4.11	-29.46	5.32	0.07
Global strain rate (per sec)					
SRe	-1.27	0.18	-1.24	0.17	0.47
SRa	2.67	0.40	2.41	0.46	0.02
SRs	0.71	0.19	0.70	0.22	0.85
Systolic strain (%)					
Base	-26.23	4.58	-24.90	5.60	0.37
Mid	-32.72	4.22	-29.27	5.74	0.02
Apical	-35.33	5.35	-33.06	6.36	0.19
Strain rate					
Base	-1.94	0.60	-1.77	0.53	0.31
Mid	-2.12	0.60	-1.93	0.53	0.26
Apical	-2.49	0.60	-2.34	0.59	0.40
Septum					
Systolic strain (%)					
Base	-20.60	2.41	-20.75	2.20	0.80
Mid	-23.29	2.16	-22.51	2.13	0.17
Apical	-25.72	3.66	-23.49	4.61	0.04
Strain rate					
Base	-1.24	0.23	-1.23	0.16	0.83
Mid	-1.37	0.18	-1.29	0.11	0.07
Apical	-1.72	0.36	-1.53	0.26	0.03

Early diastolic velocities of the right ventricle were lower in the CF children compared to the healthy controls, measured using the tricuspid inflow and tissue Doppler peak velocity of the right ventricle. The results of the deformation imaging analysis are presented in table 4. Feasibility for deformation imaging in the left and right ventricle was 87.8% and 75.7% in the CF group and 93.9% and 72.7% in the healthy group. This method showed that in almost all segments of both right and left ventricle there was a trend towards a reduced strain and strain rate in children with CF compared to healthy children. The global strain of the left ventricle in CF children was significantly lower compared to the healthy children (respectively, -21.56±2.66% versus -22.70±1.61%, p-value 0.05). The right ventricle showed a borderline significant reduction of global strain in the right ventricle (-29.46±5.32 versus -32.00±4.58%, p-value 0.07). Adjustment for weight and height or heart rate in a linear regression model, barely changed the associations.

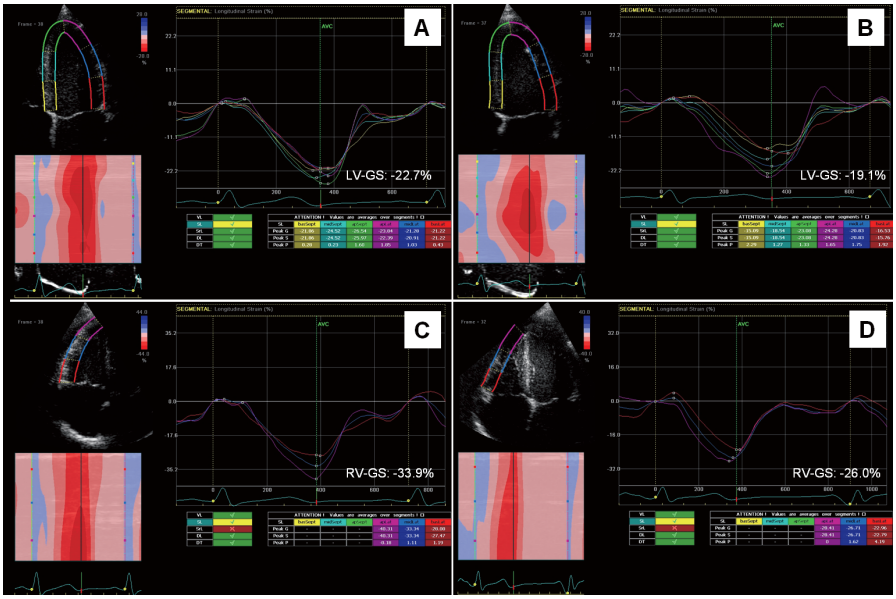

Figure 1. *Example of LV and RV deformation imaging*

This figure shows two examples of the deformation imaging analysis in the left ventricle (top) and in the right ventricle (bottom). This figure shows the slightly reduced deformation values in a child with CF (B and D) compared to a matched control subject (A and C). The characteristics of the deformation graph are normal (no dyskinetic segments, no overt post-systolic shortening), however, the amount of deformation is reduced. LV-GS: left ventricular peak systolic global strain value, RV-GS: right ventricular peak systolic global strain.

Discussion

In this study we explored the cardiovascular system in CF children with extensive measurements, including measurements of the vascular system and cardiac function of both right and left ventricle. We found evidence for subclinical changes of the cardiovascular system in clinically stable young CF patients compared to healthy children.

Previous studies in children or adolescents with CF focused on the right ventricle and had no lung function measurements of healthy reference groups.[2, 4] Using widely used measures of systolic performance of the right ventricle, TAPSE and Tissue Doppler Imaging, we saw a significant reduction in the cystic fibrosis children, while the left ventricle showed no differences in systolic performance using conventional methods or Tissue Doppler Imaging. Although parameters of global systolic function were reduced in the right ventricle when compared to the healthy reference group, the values still remained within normal limits. In addition to these methods, we assessed cardiac function of both ventricles with two-dimensional speckle tracking derived deformation imaging. This method is able to detect subclinical myocardial dysfunction in an earlier stage than the more conventional measurements.[19] Using this method, we saw a trend of a reduced performance in the different segments of both the right and left ventricle. Global strain showed a borderline significant reduction of myocardial strain of the right ventricle and a significant reduction of myocardial strain of the left ventricle in the cystic fibrosis children. The findings of the left ventricle were in line with our findings of increased arterial stiffness.

The outcome of our study regarding right ventricular dysfunction is in line with a previous reported study.[4] Left ventricular diastolic dysfunction in adult CF patients has been published before, but in these studies a large proportion of patients had severe pulmonary disease.[5, 20] It is remarkable that although there are no significant differences in lung function between our CF and healthy children, differences in cardiac function already exist at such a young age.

Several potential mechanisms to explain cardiac changes in CF have been suggested. As most studies are performed in adults with CF, many hypotheses are based on comorbidities related to CF, like cardiomyopathy as a consequence of diabetes or myocardial fibrosis due to long-standing hypoxia or subclinical ventricular dysfunction as a consequence of pulmonary hypertension. This study showed that differences in both right and left ventricular function and arterial stiffness are already present in young children with CF, who are in good clinical condition.

Aforementioned hypothesis as only explanation for the altered cardiovascular function at adult age is therefore less likely. Mechanisms that could affect the whole cardiovascular system are more plausible. Many patients with CF experience already in early life recurrent pulmonary infections, which are accompanied with higher circulating levels of pro-inflammatory mediators. These mediators could both affect cardiac contractility and arterial stiffness.[21, 22]

Arterial stiffness could also play a role in development of cardiac changes. Arterial stiffness seems to be increased in our group of children with CF, which is consistent with two previous studies performed in adults and children.[6, 7] An increase in arterial stiffness could lead to an increase in left ventricular afterload and a reduced perfusion of the coronary arteries in diastole.[7] However, we observed only subtle increase in arterial stiffness in our children with CF and it is questionable whether this increase could explain the cardiac changes entirely.

Little is known about the function of CFTR in the cardiovascular system. Experiments performed in neonatal mouse cardiomyocytes have shown that CFTR is involved in the regulation of cardiomyocyte contraction rate.[23] A more recent study in mice examined cardiac and vascular function in ΔF508

mutant mice, which do not display lung pathology.[24] Loss of CFTR function in these mice led to left ventricular remodelling and increased aortic stiffness. These findings suggest a direct effect of CFTR on cardiac changes. However, as described above, the causal mechanism of cardiac changes in CF patients remains speculative, but our findings suggest that cardiac changes are already existent early in life when longstanding and severe comorbidities are not yet present. It would be interesting to measure children in their neonatal period, to distinguish whether cardiac changes are a consequence of comorbidities of the disease or directly related to CFTR dysfunction.

Some limitations of our study have to be considered. Although, we did manage to perform elaborate cardiac measurements in 33 cystic fibrosis and 33 healthy young children, this number may still be too small to statistically detect all possible associations. However, all different parameters of myocardial strain assessment showed a trend towards an impaired strain of both the right and left ventricle in the children with cystic fibrosis. The direction of our findings are also in agreement with previous studies.[2, 4, 5] Second, this study describes only one echocardiographic evaluation in these patients. A follow-up study to correlate changes in clinical condition of the CF patients with changes in echocardiographic findings could give us more insights into the development of the cardiovascular system in these patients. Since those changes are expected to occur very slowly, this would mean a very long-term follow-up study.

With current screening and treatment options for patient with CF, life expectancy will probably further increase. With this increasing life expectancy, understanding of the development of the cardiovascular system in this patient population is needed. A progressive process of heart and vascular dysfunction may have functional significance and could generate cardiovascular disease in future adult CF patients. Furthermore, a recent study revealed that children and adolescents with CF have a higher saturated fat intake compared to healthy controls.[25] This might give an additional increased risk for cardiovascular disease later in life.

In conclusion, our study provides evidence for increased arterial stiffness and subclinical cardiac changes of both right and left ventricle in children with CF in a stable good clinical condition. These findings suggest that cardiovascular changes in CF patients are existent already in an early stage of the disease.

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Part II

Shared determinants of the cardiovascular and respiratory system in childhood

Chapter 5

Maternal BMI and cardiovascular development in childhood

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Submitted

Abstract

Background

Maternal obesity is associated with an increased risk of cardiovascular events in their adult offspring. Animal studies showed structural changes in the heart and impaired cardiac function in the offspring as a consequence of maternal obesity. Whether these findings can be extended to humans is unknown. We aimed to assess if maternal obesity is associated with impaired cardiac systolic function or cardiac dimensions in the offspring in early childhood.

Methods

Within the WHISTLER birth cohort, echocardiography was performed in 168 3-year-old children. Linear regression analyses were performed with maternal Body Mass Index (BMI) as independent variable and cardiac systolic function and dimensions as dependent variable.

Results

In all different systolic measurements there was a trend of decreasing systolic ventricular function with increasing maternal BMI. Systolic velocities derived from Tissue Doppler Imaging from the interventricular septum and right and left ventricle all reached borderline significance. There was a significant association with measurement of fractional area change of the right ventricle (-0.287 %/kg/m^2, 95% CI -0.013 to -0.561, p=0.04), even after adjustment of anthropometrics, potential confounders and pregnancy complications.

Conclusion

Higher maternal BMI may impair systolic function in healthy and non-obese offspring.

Introduction

There is an increasing prevalence of overweight and obesity among women of reproductive age. Excess weight among pregnant women is not only associated with adverse outcomes for the mother during pregnancy and in later life, but also for the offspring. A recent large Scottish cohort study described a significant association between maternal overweight or obesity and increased cardiovascular events in offspring.[1] These associations were independent of several confounders, which could reflect the prenatal and postnatal environment. This is consistent with previous findings among a cohort of Finnish men.[2] Other studies found evidence for associations between maternal or parental overweight or obesity and cardiovascular disease risk factors among the offspring, once they were adult.[3, 4] One of the cardiovascular risk factors, increased blood pressure, is also studied among offspring in childhood. Higher maternal body mass index (BMI) appeared to be associated with higher blood pressure in the offspring.[5–7]

Animal studies on maternal over-nutrition have confirmed findings of increased blood pressure in the offspring, but they also focussed on influence of maternal over-nutrition on the heart.[8–12] Structural changes in the heart like endothelial dysfunction, increased sympathetic tone, accumulation of connective tissue and myocardial fibrosis in relation with maternal over-nutrition are described.[10–12] These changes could impair the contractile function of the heart in the offspring, predisposing them to later cardiac dysfunction. This was demonstrated in a sheep model, in which fetal hearts of ewes born to obese sheep had a greater left ventricular mass and showed an impaired cardiac contractile function after a high-workload challenge compared to control fetal hearts.[11]

As far as we know, associations between maternal obesity and cardiac function in humans was not explored before and whether the findings of animal studies can be extended to humans is unknown. Since the duration of exposure to environmental and lifestyle influences is reduced in childhood, confounding by such factors is probably of minor influence in young children. Therefore, studies in young children could provide further evidence for a crucial and long lasting effect of the intrauterine environment on adverse cardiovascular health. In this study, we aimed to assess whether maternal obesity was associated with impaired cardiac systolic function and altered cardiac dimensions in the offspring in early childhood.

Methods

Setting and participants

The present study is part of the Wheezing Illnesses Study Leidsche Rijn (WHISTLER), a population-based birth cohort study on determinants and prediction of wheezing illnesses. Study design and rationale of WHISTLER were described in detail elsewhere.[13] Briefly, healthy infants born in a newly developed residential area in the Netherlands (i.e. Leidsche Rijn) were invited to participate. Exclusion criteria were gestational age <36 weeks, major congenital abnormalities and neonatal respiratory disease. From December 2011 till April 2013 we invited children at the age of 3 years for a follow-up visit.

The paediatric medical ethics committee of the University Medical Center Utrecht, the Netherlands, approved the study. Written informed consent was obtained from the parents.

Visit at the age of 3-8 weeks

Parents visited our outpatient clinic with their offspring at the age of 3-8 weeks. Information on pre- and postnatal risk factors was obtained by questionnaires. Weight of the child was measured using a standard electronic scale and body length using an infant stadiometer. Information of the parents was obtained from the linked database of the Utrecht Health Project (UHP), a large health monitoring study of all inhabitants of Leidsche Rijn.[14] In total, 44 fathers and 52 mothers of the 168 children of this study participated in the UHP. These parents participated in UHP before pregnancy. Weight and height of these participants were measured using a standard electronic scale and body length using a stadiometer. The parents who did not participate in the UHP were asked to fill in a questionnaire about their health, including self-reported weight and height.

Visit at the age of 3 years

A health questionnaire containing information about risk factors and health status of both the participant and parents was completed by the parents prior to this follow-up visit. This questionnaire included a question about current weight and height of the parents as well. During the visit, weight of the children was measured in light clothing using a standard electronic scale and height without wearing shoes using a stadiometer.

Transthoracic echocardiographic imaging was performed using a ViVid 7 ultrasound machine (GE Healthcare, Milwaukee, WI, Wauwatosa, U.S.A.) with a 6S, 2.7-8.0 MHz transducer with continuous electrocardiographic monitoring. All measurements were performed at rest without using sedation, with the children lying in a supine left lateral position. All echocardiograms were performed by the same observer (JE) and analysed by another observer (MW). In 96.4% (162/168) of the children we could perform echocardiography measurements. If the child refused to participate we stopped the measurements. All measurements were performed using the recommendations of the American Society of Echocardiography.[15, 16]

Covariates

Covariates that could mediate or confound the main associations of interest were selected a priori based on previous literature. We adjusted for several potential confounders, including maternal smoke exposure during pregnancy, current smoke exposure of the child (at the 3-year-visit), socio-economic status based on maternal education and duration of breastfeeding. Furthermore, we adjusted for complications or procedures during pregnancy or delivery, which could mediate our association of interest. These covariates included hypertension, diabetes, use of antibiotics and infections during pregnancy and mode of delivery.

Statistical analysis

Central estimators and variance measures to describe general characteristics were calculated. All variables were checked for normality of distribution. Differences in characteristics of pregnancy and of the offspring across quartiles of increasing maternal BMI were tested using Analysis Of Variance (ANOVA) for continuous variables and χ^2 for categorical variables. To assess if we could use current maternal BMI as a proxy for prepregnancy BMI we used Pearson's correlation coefficients to examine the relation between BMI measured in UHP before pregnancy, self-reported maternal BMI 3-8 weeks after delivery and self-reported maternal BMI of the visit when the child was 3-years-old.

The relation between maternal BMI and cardiac function was assessed using linear regression analyses, with maternal BMI as independent variable and systolic and diastolic function and dimensions of the heart as dependent variables. Using multivariable regression models we adjusted for anthropometrics, potential confounders and complications during pregnancy. Statistical significance was considered reached at p-value <0.05. All analyses were performed with SPSS version 20.0 for Windows (IBM, Armonk, New York, USA).

Results

Table 1 presents the baseline characteristics per BMI quartile. The mean maternal BMI from the lowest to highest quartile was respectively 20.2 kg, 22,5 kg, 24,8 kg and 30.1 kg. There were trends of more common hypertension in the mothers (p=value 0.07) and more use of antibiotics (p-value 0.06) with increasing maternal BMI. During pregnancy, mothers with a higher BMI experienced more infections (p-value 0.04). In total, 21.1% of the mothers in the highest quartile were exposed to cigarette smoke during pregnancy, more than in the other BMI groups (p-value 0.02). Rates of caesarean delivery tended to increase with increasing quartile of maternal BMI (p-value 0.07). There was no difference in environmental factors or anthropometrics of the child.

There was a strong correlation between self-reported maternal BMI at the neonatal and child age 3-year visits (correlation coefficient 0.89, p-value <0.001) and between the self-reported maternal BMI at the child age 3-years visit and actually measured maternal BMI before pregnancy (correlation coefficient 0.84, p-value <0.001).

Table 2 shows the result of the main analysis with indicators of cardiac systolic function. In all different systolic measurements there was a trend of decreasing systolic left and right ventricular function with increasing maternal BMI. Fractional shortening of the left ventricle showed no clear association, but systolic velocities derived from Tissue Doppler Imaging of the interventricular septum and of the left ventricle reached borderline significance. However, in the left ventricle in model 3, after adjustment for potential confounders peak systolic velocity tended to be lower with increasing maternal BMI (-0.067 cm/sec per kg/m^2, 95% CI -0.141, 0.006, p=0.07).

Table 1. *Pregnancy and child characteristics by quartiles of maternal BMI*

		Maternal BMI per quartile								
		1		2		3		4		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value
General characteristics										
Maternal BMI (kg/m^2)		20.2	0.9	22.5	0.7	24.8	1.0	30.1	3.2	<0.01
Sex (n,%)	Male	15	38.5	20	47.6	24	58.5	15	37.5	0.20
	Female	24	61.5	22	52.4	17	41.5	25	62.5	
Older siblings (n,%)	No	19	48.7	19	45.2	16	39.0	17	43.6	0.85
	Yes	20	51.3	23	54.8	25	61.0	22	56.4	
Maternal education (n,%)	Other	9	26.5	7	21.2	9	26.5	8	21.6	0.92
	High*	25	73.5	26	78.8	25	73.5	29	78.4	
Characteristics of pregnancy										
Hypertension during pregnancy (n,%)	No	34	91.9	37	94.9	37	94.9	31	79.5	0.07
	Yes	3	8.1	2	5.1	2	5.1	8	20.5	
Antibiotics during pregnancy (n,%)	No	21	100.0	21	91.3	18	78.3	21	75.0	0.06
	Yes	0	0.0	2	8.7	5	21.7	7	25.0	
Diabetes during pregnancy (n,%)	No	37	100.0	39	100.0	38	97.4	37	94.9	0.30
	Yes	0	0.0	0	0.0	1	2.6	2	5.1	
Infections during pregnancy (n,%)	No	36	97.3	36	92.3	33	84.6	30	76.9	0.04
	Yes	1	2.7	3	7.7	6	15.4	9	23.1	
Smoke exposure pregnancy (n,%)	No	35	89.7	42	100.0	37	90.2	30	78.9	0.02
	Yes	4	10.3	0	0.0	4	9.8	8	21.1	
Delivery (n,%)	Normal	32	91.4	32	82.1	31	79.5	26	66.7	0.07
	Caesarean	3	8.6	7	17.9	8	20.5	13	33.3	
Characteristics child 1st year										
Gestational age (days)		278	11	276	10	279	8	275	9	0.25
Birth weight (gr)		3620	431	3491	438	3671	468	3605	623	0.40
Birth length (cm)		51	2	51	2	51	2	51	2	0.71
Weight growth (gr/day)		28.6	6.4	29.3	5.4	29.9	4.1	27.2	5.9	0.15
Length growth (cm/day)		0.12	0.01	0.12	0.01	0.12	0.01	0.12	0.01	0.16
Day care 1st year of life (n,%)	No	12	30.8	9	21.4	11	26.8	7	17.5	0.79
	1-3 months	2	5.1	4	9.5	3	7.3	4	10.0	
	4-6 months	2	5.1	6	14.3	2	4.9	4	10.0	
	>6 months	23	59.0	23	54.8	25	61.0	25	62.5	
Characteristics child 3rd years										
Age (years)		3.75	0.16	3.70	0.16	3.72	0.17	3.71	0.17	0.46
Weight (kg)		17.0	2.4	17.1	1.7	17.4	2.2	17.3	2.9	0.90
Height (cm)		103	5	101	4	103	4	102	5	0.50
Postnatal smoke exposure (n,%)	No	37	94.9	41	97.6	39	95.1	37	92.5	0.52
	Yes	2	5.1	1	2.4	2	4.9	3	7.5	

Values are means and standard deviations (SD) unless otherwise indicated. *Higher vocational or university education. Weight and height growth during the first three months of life.

Table 2. *Associations between maternal BMI and cardiac systolic function in the offspring*

	N	Linear regression coefficient	95% Confidence Interval	p-value
Left ventricle				
Fractional shortening (%)				
Model 1	151	-0.042	-0.176, 0.091	0.531
Model 2	151	-0.037	-0.172, 0.099	0.593
Model 3	129	-0.078	-0.231, 0.074	0.311
Model 4	141	0.005	-0.139, 0.148	0.949
TDI S peak systolic velocity (cm/sec)				
Model 1	148	-0.045	-0.110, 0.020	0.175
Model 2	148	-0.038	-0.102, 0.027	0.248
Model 3	125	-0.067	-0.141, 0.006	0.073
Model 4	139	-0.045	-0.116, 0.026	0.212
Interventricular septum				
TDI S peak systolic velocity (cm/sec)				
Model 1	153	-0.025	-0.055, 0.005	0.106
Model 2	153	-0.027	-0.057, 0.004	0.087
Model 3	130	-0.026	-0.058, 0.006	0.117
Model 4	143	-0.025	-0.057, 0.008	0.132
Right ventricle				
TDI S peak systolic velocity (cm/sec)				
Model 1	150	-0.068	-0.141, 0.005	0.066
Model 2	150	-0.063	-0.136, 0.010	0.090
Model 3	125	-0.075	-0.157, 0.006	0.070
Model 4	141	-0.060	-0.138, 0.018	0.129
RV FAC (%)				
Model 1	147	-0.287	-0.561, -0.013	0.040
Model 2	147	-0.293	-0.571, -0.015	0.039
Model 3	122	-0.287	,-0.013, -0.561	0.040
Model 4	139	-0.359	-0.651, -0.068	0.016
TAPSE (mm)				
Model 1	150	-0.035	-0.114, 0.045	0.309
Model 2	150	-0.026	-0.104, 0.051	0.502
Model 3	126	-0.064	-0.157, 0.028	0.170
Model 4	142	-0.020	-0.105, 0.065	0.645

TDI: Tissue Doppler Imaging, S: Systolic, RV FAC: right ventricle fractional area change, TAPSE: Tricuspid Annular Plane Systolic Excursion.
Model 1: Unadjusted
Model 2: Weight, Height
Model 3: Current smoking, smoking during pregnancy, breastfeeding, socio-economic status
Model 4: Delivery, infections during pregnancy, hypertension during pregnancy

Table 3. *Association between maternal BMI and dimensions of the heart in the offspring*

	N	Linear regression coefficient	95% Confidence Interval	p-value
IVSd (mm)				
Model 1	151	-0.042	-0.081, -0.0003	0.033
Model 2	151	-0.038	-0.076, 0.000	0.053
Model 3	129	-0.056	-0.100, -0.012	0.013
Model 4	141	-0.034	-0.075, 0.008	0.108
LVEDD (mm)				
Model 1	151	-0.050	-0.165, 0.064	0.388
Model 2	151	-0.048	-0.154, 0.058	0.375
Model 3	129	-0.073	-0.206, 0.059	0.276
Model 4	141	-0.031	-0.152, 0.091	0.618
LVPWd (mm)				
Model 1	151	0.008	-0.029, 0.044	0.681
Model 2	151	0.009	-0.027, 0.046	0.608
Model 3	129	-0.003	-0.044, 0.039	0.889
Model 4	141	0.006	-0.033, 0.046	0.758
LVESD (mm)				
Model 1	151	-0.008	-0.097, 0.082	0.865
Model 2	151	-0.008	-0.094, 0.079	0.861
Model 3	125	-0.010	-0.114, 0.094	0.846
Model 4	141	-0.011	-0.107, 0.086	0.827
RV diameter (mm)				
Model 1	148	0.021	-0.112, 0.155	0.752
Model 2	148	0.009	-0.119, 0.138	0.886
Model 3	135	0.014	-0.139, 0.167	0.852
Model 4	138	-0.014	-0.152, 0.123	0.838
RV length (mm)				
Model 1	147	-0.033	-0.202, 0.135	0.696
Model 2	147	-0.027	-0.186, 0.132	0.734
Model 3	134	-0.126	-0.317, 0.065	0.195
Model 4	137	0.004	-0.176, 0.184	0.965

IVSd: interventricular septum in diastole, LVEDD: left ventricular end-diastolic dimension, LVPWd: left ventricular posterior wall in diastole, LVESD: left ventricular end-systolic dimension, RV diameter: right ventricular diameter, RV length: right ventricular length.

Model 1: Unadjusted
Model 2: Weight, Height
Model 3: Current smoking, smoking during pregnancy, breastfeeding, socio-economic status
Model 4: Delivery, infections during pregnancy, hypertension during pregnancy

Systolic velocities of the right ventricle measured with Tissue Doppler were borderline significant as well (unadjusted model: -0.07 cm/sec per kg/m^2, 95% CI -0.141, 0.005). Although no associations were observed with tricuspid annular plane systolic excursion (TAPSE) there was a significant association with measurement of fractional area change of the right ventricle (linear regression coefficient -0.287 %/kg/m^2, 95% CI -0.013, -0.561), even after adjustment of anthropometrics (model 2), potential confounders (model 3) and pregnancy complications (model 4).

Table 3 shows the results of main analysis with cardiac dimensions. With each kg/m^2 increase in maternal BMI, the interventricular septum thickness decreased with 0.06 mm (95% CI -0.100, -0.012, p=0.013). There was no significant association or clear trend with other measures of dimensions of the heart.
We observed no association between maternal BMI and diastolic function of the left ventricle (see supplementary table 1).

Discussion

In this first study in humans exploring associations between maternal BMI and their offspring's cardiac function and structure, we observed a trend of reduced systolic function with increasing maternal BMI. All but one of the associations between maternal BMI and parameters of ventricular function were negative in direction. Although subtle, this finding suggests an association between higher maternal BMI and an impaired systolic function in the offspring, measured in early childhood, which is in line with animal studies.[11, 17]
Our prospective birth cohort is embedded in the UHP. Almost one third of the parents are also participants in that study. This infrastructure enabled us to correlate self-reported weight and height at the 3-years visit with actually measured pre-pregnancy BMI and provided detailed information of many potential confounders and mediating factors to be used in the present study. By studying our children in early childhood we have tried to minimize influences of postnatal lifestyle and environmental factors. Two recent studies investigating associations between parental body mass index and risk factors for cardiovascular disease among their offspring in mid-life showed that the associations described were largely explained by offspring adiposity.[3, 4] In our study, we adjusted for current weight and height of the child (model 2). This did not change our findings. This could mean that effects of higher maternal BMI on offspring cardiac function precede any manifestation of a familial propensity for offspring to become overweight or obese in later life. Adjustments for other potential confounders did not change the findings as well. Although our data suggest a direct effect and negative effect of maternal BMI on ventricular function in early childhood, the challenge remains to disentangle shared genetics, postnatal lifestyle and environmental factors from the direct effects of maternal obesity. Building on previous evidence showing more cardiovascular risk factors and events in adult offspring of mothers with high BMI,[1–4] we postulate that the diminished ventricular function at young age is a predictor – maybe causative factor – in the later cardiovascular events.

Several hypotheses could be formulated to explain the mechanism of the association between maternal BMI and cardiac function in the offspring. Maternal obesity induces rises in fetal hormones, like leptin and insulin, nutrients (fatty acids, triglycerides and glucose) and inflammatory cytokines.[17] They could all play a role in the development of the heart in the offspring. In sheep, they observed that maternal obesity induced inflammation in fetal skeletal muscle.[11] In a subsequent study the same investigators demonstrated that maternal obesity resulted in greater fetal heart connective tissue accumulation associated with an upregulated TGF-β and p38 signalling pathway at late gestation.[10] Evidence from animals is all derived from over-nutrition models, it is unknown whether findings of these models can be extrapolated to maternal obesity in humans, although our findings in human offspring in early childhood do seem to largely correspond.

In adults with obesity or chronic hypertension, the first signs of cardiac dysfunction are subtle changes in diastolic function.[18] Previous studies reported higher blood pressure during childhood and adulthood among offspring of mothers with a higher maternal BMI. In our children we saw evidence for systolic function and no evidence for diastolic dysfunction of the left ventricle. This is in line with a study in sheep, which showed that under high-workload, the velocity of cardiac systolic contraction was impaired in fetal hearts of ewes of an obese sheep, while there was no difference in diastolic contraction compared to control fetal hearts.[11] Although long-term exposure to overweight, and closely related high blood pressure, may affect cardiac function in their own right, our data suggest that the first detrimental cardiac effects of familial propensity to overweight can be detected in healthy infancy, well before such propensity becomes manifest.

There are limitations of our study. First, we used current maternal BMI reported at the 3-years-visit as surrogate of BMI before pregnancy. Self-reported weight and height might result in some misclassification of BMI. Mothers with higher weight might be expected to underreport their weight, which could lead to dilution bias in our estimates. However, although we used current maternal BMI, we were able to correlate these with self-reported BMI at the neonatal visit and even with BMI of 1/3 of the mothers whose weight and height were actually measured before pregnancy. There was a strong correlation between these different measures. Second, the decrease in septum thickness with increasing maternal BMI that we found is not consistent with animal studies that demonstrated an increased left ventricular mass in offspring of obese ewes and not with all other cardiac dimensions in our study, which showed no trend of associations with maternal BMI. We do not have an explanation for this finding.

We are the first exploring this association in humans and, as expected, the impact of maternal obesity on systolic function is subtle. We performed elaborate cardiac measurements in almost 170 healthy preschool children. This number may still be too small to statistically detect all possible associations. Therefore, replication and validation of our findings in larger cohorts is warranted. The consequences of our findings remain yet unclear. Many cardiovascular risk factors, including blood pressure, track into adulthood.[19, 20] Whether this is also true for the subtle changes in cardiac function that we found is unclear, but we think it is reasonable to assume that cardiac function tracks into adulthood as well. Therefore, a small decrease in systolic function in childhood may have a larger impact on cardiovascular

disease in later life. Our study adds to and extends accumulating evidence for the developmental origins of health and disease hypothesis, which proposes that adverse conditions in utero might lead to lifelong changes in body composition and physiology, with could lead to adverse health in adulthood.

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Supplementary table 1. *Association between maternal BMI and diastolic function of the left ventricle in the offspring.*

	N	Linear regression coefficient	95% Confidence Interval	p-value
MV E (m/s)				
Model 1	149	-0.005	-0.010, 0.001	0.083
Model 2	149	-0.005	-0.011, 0.000	0.071
Model 3	125	-0.004	-0.011, 0.002	0.150
Model 4	139	-0.003	-0.009, 0.003	0.288
MV A (m/s)				
Model 1	149	-0.001	-0.005, 0.003	0.680
Model 2	149	-0.001	-0.006, 0.003	0.582
Model 3	125	0.000	-0.004, 0.005	0.959
Model 4	139	0.000	-0.005, 0.004	0.940
MV E/A				
Model 1	149	-0.008	-0.021, 0.006	0.269
Model 2	149	-0.007	-0.021, 0.006	0.297
Model 3	125	-0.010	-0.026, 0.005	0.186
Model 4	139	-0.007	-0.021, 0.008	0.365
TDI LV lateral wall e' (cm/s)				
Model 1	148	0.073	-0.047, 0.192	0.231
Model 2	148	0.081	-0.039, 0.202	0.185
Model 3	125	0.065	-0.075, 0.205	0.360
Model 4	139	0.061	-0.066, 0.189	0.343
TDI LV lateral wall a' (cm/s)				
Model 1	144	-0.036	-0.094, 0.022	0.223
Model 2	144	-0.042	-0.099, 0.015	0.143
Model 3	121	0.065	-0.075, 0.205	0.360
Model 4	136	-0.024	-0.086, 0.038	0.454
TDI septal e' (cm/s)				
Model 1	153	0.049	-0.012, 0.111	0.117
Model 2	153	0.053	-0.009, 0.115	0.093
Model 3	130	0.047	-0.021, 0.114	0.172
Model 4	143	0.045	-0.021, 0.110	0.180
TDI septal a' (cm/s)				
Model 1	153	-0.001	-0.048, 0.045	0.952
Model 2	153	-0.005	-0.052, 0.043	0.839
Model 3	130	0.001	-0.052, 0.054	0.968
Model 4	143	-0.006	-0.054, 0.042	0.795

MV: Mitral valve, E: early diastolic velocity, A: late diastolic velocity, LV: left ventricular, TDI: Tissue Doppler Imaging.

Model 1: Univariable
Model 2: Weight, Height
Model 3: Current smoking, smoking during pregnancy, breastfeeding, socio-economic status
Model 4: Delivery, infections during pregnancy, hypertension during pregnancy

Chapter 6

Maternal body mass index, neonatal lung function and respiratory symptoms in childhood

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Submitted

Abstract

Background

Recent studies have shown that maternal obesity is associated with increased risk of wheezing in the offspring. We assessed if impaired neonatal lung function could explain this association.

Methods

We measured neonatal lung function in 2606 children of our birth cohort (WHISTLER). Information about daily symptoms of wheezing in the first year of life was obtained from questionnaires. Consultations and prescriptions for wheezing illnesses were derived from patient files of the general practitioner.

Results

Higher maternal BMI was associated with increased risk of wheezing in the first year of life and more consultations and prescriptions for wheezing illnesses till age 5. Lung function could partially explain the association with wheezing in the first year of life. Adding resistance to the model decreased the incidence rate ratio from 1.023 (95% CI 1.008-1.039) to 1.014 (0.997-1.032). Current weight and height of the 5-year-olds largely explained the association with consultations. Intermediates or confounders could not explain the association with prescriptions.

Conclusion

The association between a higher maternal BMI and increased risk of wheezing the first year of life is partially explained by an impaired lung function in early life. At the age of 5 years infant lung function is of minor influence.

Introduction

Maternal pre-pregnancy obesity is associated with adverse health outcomes for the offspring. Children of mothers with a higher body mass index (BMI) have an increased risk of obesity, high blood pressure and diabetes mellitus type 2 in childhood and even long-term consequences on cardiovascular health are described.[1–4] With an increasing prevalence of both obesity and asthma, a growing interest has recently arisen in the association between maternal weight and respiratory symptoms.[5–8] Several studies have shown that increased maternal weight and BMI are associated with an increased risk of wheezing in offspring in the first years of life, independent of several confounders.[5, 6, 8] Associations of maternal BMI and asthma symptoms in later childhood and adolescence are less clear. The underlying mechanism of these associations is unknown. The association could not be explained by the child's growth, infectious or atopic mechanisms and was also not mediated by obesity-related pregnancy complications.[5, 9]

Previous studies suggested that leptin, a hormone that is primarily produced by adipocytes, could play a role in the mechanism underlying these associations.[7, 9] Leptin receptors have been identified in the human bronchial and alveolar epithelial cells, bronchial smooth muscle cells, and bronchial submucosa. In a recent study we have shown an association between higher leptin plasma concentration and lower lung function in healthy children, which suggests a functional role of this hormone in the respiratory system.[10] Children of mothers with increased fat mass are probably exposed to higher circulating levels of leptin, which may have consequences for development of the respiratory system. Children with an impaired lung function in early life are more susceptible to develop wheezing illnesses.

We examined in our population-based prospective birth cohort study the association between maternal BMI and respiratory symptoms and the use of medication for respiratory symptoms in the offspring and assessed if early life lung function could be an explaining factor in these associations.

Methods

Setting and participants

The present study is part of the Wheezing Illnesses Study Leidsche Rijn (WHISTLER), a population-based birth cohort study on determinants and prediction of wheezing illnesses. Study design and rationale of WHISTLER were described in detail elsewhere.[11] Briefly, healthy infants born in a newly developed residential area in the Netherlands (i.e. Leidsche Rijn) were invited to participate. Exclusion criteria were gestational age <36 weeks, major congenital abnormalities and neonatal respiratory disease.

The paediatric medical ethics committee of the University Medical Center Utrecht, the Netherlands, approved the study. Written informed consent was obtained from the parents.

Visit in infancy

Parents visited our outpatient clinic with their offspring at the age of 3-8 weeks. Information on pre- and postnatal risk factors was obtained by questionnaires. Weight of the child was measured using a standard electronic scale and body length using an (infant) stadiometer. Information of the parents

was obtained from the Utrecht Health Project (UHP), a large health monitoring study of all inhabitants of Leidsche Rijn.[12] In total 969 fathers and 1128 mothers of the 2686 children participated in this study. The parents who did not participate in the UHP were asked to fill in a questionnaire about their health, including self-reported weight and height.

Lung function was measured using the single occlusion technique (SOT) during natural sleep. It measures the resistance (Rrs), compliance (Crs) and time constant (trs) of the total respiratory system in the absence of respiratory muscle activity.[13] Details of performance of these measurements were previously described.[13, 14] Measurements were performed according to the criteria of the American Thoracic Society (ATS)/European Respiratory Society (ERS) Task Force on infant lung function.[15, 16] At least three technically acceptable occlusions were used to calculate mean Crs, Rrs and trs. Interrater reliability studies were performed as part of Whistler and showed satisfactory quality of lung function measurements.[14]

Follow-up data first year

One-year follow-up for symptoms of wheeze and cough after infant lung function measurement was achieved by a daily questionnaire completed by the parents in a logbook. Parents were carefully instructed at the time of lung function measurements by one of the investigators on how to recognise the various respiratory sounds. Daily complaints of wheeze and cough were measured using the questions: "Did your child wheeze today (whistling sound from the chest, not from the upper airways/throat)?". Further questions were asked about anthropometrics and environmental factors, such as feeding pattern, passive smoking and day care attendance. New questionnaires and reinforcements to complete them were sent on a monthly basis to the parents. If parents still failed to return the questionnaire, they were contacted by telephone. To quantify respiratory symptoms, number of days with wheeze in their first year of life was counted.

Visit at the age of 5 years

At the age of 5 years the children were invited for a follow-up visit. Prior to this visit, parents were asked to complete a questionnaire about current health and risk factors of their child. During the visit, weight and height were measured and spirometry was performed according to the latest American Thoracic Society (ATS)/ European Respiratory Society (ERS) statement for lung function measurements in preschoolers.[17] At least two reproducible flow-volume curves were obtained. The largest forced expiratory volume in 1 second (FEV1) was selected.

Medication use and diagnosis of respiratory symptoms

Data on primary care visits and prescriptions from birth until the age of 5 years were obtained from the general practitioners' (GP) electronic patient files (Medicom, PharmaPartners, the Netherlands), using standardized codes. The general practitioners used the International Classification of Primary Care (ICPC)

codes for every consultation. The following ICPC codes were used for a diagnosis of lower respiratory symptoms: dyspnea (R02); wheezing (R03); cough (R05); acute bronchitis/bronchiolitis (R78); pneumonia (R81); asthma (R96). The ICPC codes dyspnea (R02), wheezing (R03) and asthma (R96) were used for a diagnosis of wheezing illnesses. Medication was classified according to the Anatomical Therapeutical Chemical (ATC) classification. To quantify consultations and prescriptions for respiratory illnesses, number of diagnosis of lower respiratory symptoms and wheezing illnesses and number of prescriptions for short-acting β2 agonists and inhaled corticosteroids until the age of 5 years were counted.

Statistical analysis

Central estimators and variance measures to describe general characteristics were calculated. All variables were checked for normality of distribution. Differences in characteristics of pregnancy and of the offspring across quartiles of increasing maternal BMI were tested using Analysis Of Variance (ANOVA) for continuous variables and χ^2 for categorical variables.

The number of days with wheezing between 2nd and 12th month of age was used as a count type outcome, best fitting a negative binomial distribution, as there were many children with no days of wheezing symptoms. We constructed a univariable model to investigate the relation between maternal BMI and number of days with wheeze. Subsequently, multivariable negative binomial regression models were constructed to investigate if maternal BMI was independently related to number of days with wheeze or cough. We adjusted for intermediates or potential confounders. Selection of confounders was based on previous literature. Infant lung function was added to a model with potential confounders to examine if lung function could explain an association between maternal BMI and wheezing illnesses. Selective loss to follow-up could bias our results, as returning questionnaires every month requires effort of the parents. To prevent bias associated with missing data, missing values of determinants and the outcome were multiple imputed based on the correlation of the missing variables with other characteristics. Ten imputed data sets were created and analysed separately, after which results were pooled. We performed the same negative binomial analysis on the imputed data.

The number of consultations and prescriptions till the age of 5 years were also used as a count type, best fitting a negative binomial distribution. We performed similar multivariable regression models with these data.

Finally, we assessed if maternal atopy could be an effect modifier of this association. Therefore, we stratified the data by maternal atopy and performed the same analyses as described above. To test if modification was significant we added the product of maternal atopy and BMI as interaction term to the multivariable regression models.

Results are presented as incidence rate ratio (IRR) with their 95% confidence interval (CI) and p-values. Intervals not including 1 and p-values <0.05 were considered statistically significant. Data analyses were performed in SPSS version 20.0.

Table 1. *Pregnancy and child characteristics by quartiles of maternal BMI*

		Maternal BMI per quartile								
		1		2		3		4		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value
Maternal and pregnancy characteristics										
Maternal BMI (kg/m^2)		20.5	1.1	23.0	0.6	25.2	0.8	30.0	3.2	<0.01
Maternal age at birth (years)		32.7	3.7	32.7	3.6	32.8	3.6	33.0	3.8	0.52
Gestational age (days)		278.3	9.6	279.2	9.6	278.2	10.0	278.9	9.8	0.29
High maternal education* (n, %)		384	69.1	400	74.5	390	71.2	319	59.7	0.52
Maternal atopy (n, %)		202	36.1	208	37.7	196	35.4	218	39.4	0.52
Maternal western ethnicity (n, %)		436	78.0	422	76.3	461	82.9	447	81.1	0.03
Smoke exposure during pregnancy (n,%)		57	10.1	63	11.2	69	12.3	96	17.1	<0.01
Child characteristics of neonatal visit (n=2257)										
Female sex (n, %)		303	53.6	292	51.9	280	49.6	276	48.9	0.37
Birth weight (gr)		3451	475	3528	485	3512	499	3634	542	<0.01
Birth length (cm)		50.6	2.2	50.9	2.2	50.8	2.4	51.1	2.3	<0.01
Weight growth during the first three months of life (gr/day)		28.8	5.2	29.2	5.6	28.8	5.6	28.9	5.2	0.71
Length growth during the first three months of life (cm/day)		0.12	0.01	0.12	0.01	0.12	0.01	0.12	0.01	0.30
Compliance of the respiratory system (mL/kPa)		46.5	11.0	46.9	11.0	46.0	11.3	47.0	12.0	0.51
Resistance of the respiratory system (kPa/L/s)		6.2	2.1	6.6	2.1	6.7	1.9	6.7	2.0	0.69
Older siblings (n, %)		312	55.3	302	53.7	319	56.9	296	52.7	0.52
Day care during the first year of life (n,%)	No	153	29.2	132	26.2	120	23.3	151	30.1	0.12
	0-3 months	29	5.5	24	4.8	23	4.5	21	4.2	
	4-6 months	59	11.3	50	9.9	49	9.5	37	7.4	
	>6 months	283	54.0	297	59.0	323	62.7	293	58.4	
Duration of breastfeeding (n, %)	No	102	18.4	96	17.4	108	19.6	163	29.5	<0.01
	0-3 months	237	42.9	258	46.8	258	46.7	244	44.2	
	4-6 months	119	21.5	140	25.4	110	19.9	91	16.5	
	>6 months	95	17.2	57	10.3	76	13.8	54	9.8	
Pet keeping (n, %)		196	34.7	206	36.6	217	38.4	225	39.9	0.30
Child characteristics 5-years visit (n=680)										
Weight		19.4	2.3	20.1	2.5	20.3	2.8	20.7	3.1	<0.01
Height		114.8	5.0	115.6	4.6	115.5	5.2	115.1	4.6	0.48
FEV1 (liters)		1.24	0.20	1.25	0.23	1.31	0.76	1.29	0.26	0.35

Values are means and standard deviations (SD) unless otherwise indicated. *Higher vocational or university education.

Results

An overview of the recruitment and inclusion of participants of the WHISTLER-project is given in figure 1. In total, 2686 children participated within Whistler. In total, 75.0% of all eligible infants completed the monthly questionnaires till the age of 1 year. Data of consultations and prescriptions were available of 76.1% of all children who reached the age of 5 years. To detect possible confounders, table 1 presents the baseline characteristics of these children per BMI quartile. The mean maternal BMI in the first, second, third and fourth quartile was respectively 20.5 kg, 23.0 kg, 25.2 kg and 30.0 kg. Mothers with a higher BMI were more exposed to smoke during pregnancy (p-value <0.01) and were more often of western origin (p-value 0.03). Infants of mothers with a higher maternal BMI had a higher height (at birth) and weight at birth and at the visit at the age of 5 years (all p-values <0.01). Children of mothers in the highest quartile were less exposed to breastfeeding (p-value <0.01). There were no differences in postnatal growth, lung function or other environmental factors.

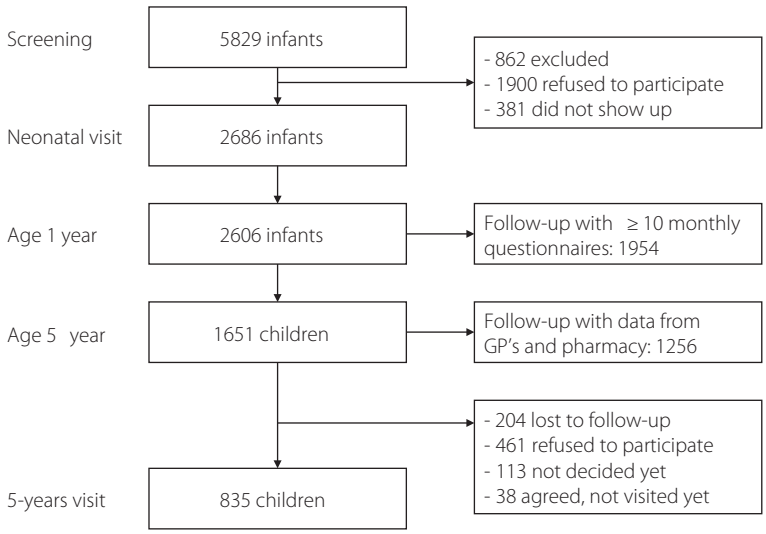

Figure 1. *Overview of the study population (May 2013)*

Table 2 shows the results of the negative binomial regression analysis with number of wheezing days in the first year of life as dependent variable. In the complete case analyses, every kg/m^2 increase in maternal BMI was associated with 2.3% more wheezing days (95% confidence interval (95% CI) 1.008-1.039) after adjustment for potential confounders. Birth weight did not explain the association. Adding resistance of the respiratory system decreased the IRR to 1.014, not statistically significant anymore (95% CI 0.997-1.032). Although the univariable model of the imputed data showed no clear association between maternal BMI and number of wheezing days (IRR 1.008, 95% CI 0.988-1.029), the multivariable models showed similar results compared to the complete case analysis. Adding resistance to the model

with potential confounders decreased the IRR from 1.019 (95% CI 1.005-1.035) to 1.004 (95% CI 0.998-1.021).

Table 2. *Association between maternal BMI and number of wheezing days in the offspring*

	Complete cases				Imputed data		
	N	IRR	95% CI	p	IRR	95% CI	p
1	1762	1.018	1.004, 1.032	0.01	1.008	0.988, 1.029	0.40
2	1652	1.023	1.008, 1.039	<0.01	1.019	1.005, 1.035	0.01
3	1651	1.028	1.012, 1.045	<0.01	1.022	1.006, 1.038	0.01
4	1401	1.038	1.021, 1.056	<0.01	1.020	1.005, 1.035	0.01
5	1401	1.014	0.997, 1.032	0.10	1.004	0.988, 1.021	0.63

1. Univariable
2. Day care, breastfeeding, older siblings, smoke exposure during pregnancy, pet keeping, socio-economic status, ethnicity, maternal age
3. All variables of model 2 and birth weight
4. All variables of model 2 and CRS
5. All variables of model 2 and RRS

Table 3. *Association between maternal BMI and number of consultations and prescriptions for wheezing illnesses in the offspring*

	Consultations for Wheezing illnesses				Prescriptions of Inhaled corticosteroids				Prescriptions of Short-acting β2 agonists		
Model	N	IRR	95% CI	p	N	IRR	95% CI	p	IRR	95% CI	p
1	1033	1.035	1.009, 1.062	0.01	1051	1.076	1.047, 1.106	<0.01	1.046	1.023, 1.069	<0.01
2	873	1.033	1.002, 1.066	0.04	888	1.098	1.063, 1.134	<0.01	1.059	1.032, 1.085	<0.01
3	524	1.004	0.964, 1.046	0.84	524	1.110	1.061, 1.160	<0.01	1.076	1.038, 1.115	<0.01
4	705	1.022	0.986, 1.059	0.23	717	1.078	1.037, 1.121	<0.01	1.058	1.028, 1.088	<0.01

1. Univariable
2. Day care, breastfeeding, older siblings, smoke exposure during pregnancy, pet keeping, socio-economic status, ethnicity, maternal age
3. All variables of model 2 and weight and height at 5 years visit
4. All variables of model 2 and RRS

Table 3 shows the association between maternal BMI and consultations and prescriptions for respiratory symptoms. With each increase in kg/m^2 maternal BMI 3.3% (95% CI 1.002-1.066) more consultations for wheezing illnesses were present after adjustment for potential confounders. Current weight and height of the child at the age of 5 years explained the association with wheezing illnesses (IRR 1.004, 95% CI 0.964- 1.046). Lung function in early life could partially explain the association with wheezing illnesses (IRR 1.022 (95% CI 0.986- 1.059). Higher maternal BMI was also associated with more prescriptions of short-acting β2 agonists and inhaled corticosteroids in the offspring. These associations could not be explained by potential confounders, current status of weight and height or lung function in early life.

Table 4. *Association between maternal BMI and number of consultations and prescriptions for wheezing illnesses in the offspring, stratified by maternal atopy*

	No maternal atopy				Maternal atopy				
Model	**N**	**IRR**	**95% CI**	**p**	**N**	**IRR**	**95% CI**	**p**	**p-value interaction**
Wheezing illnesses									
1	629	1.007	0.973, 1.043	0.69	373	1.070	1.027, 1.115	<0.01	<0.01
2	549	0.997	0.955, 1.040	0.88	322	1.048	0.995, 1.104	0.08	0.04
3	331	0.940	0.883, 1.002	0.06	192	1.029	0.959, 1.105	0.43	0.02
4	442	0.986	0.938, 1.036	0.57	261	1.047	0.987, 1.111	0.13	0.02
Inhaled corticosteroids									
1	638	1.050	1.012, 1.090	0.01	382	1.113	1.066, 1.161	<0.01	<0.01
2	557	1.055	1.011, 1.102	0.01	329	1.111	1.053, 1.173	<0.01	0.05
3	335	1.024	0.960, 1.093	0.47	197	1.125	1.040, 1.216	<0.01	0.12
4	447	1.007	0.952, 1.065	0.81	268	1.115	1.048, 1.187	<0.01	<0.01
Short-acting β2 agonists									
1	638	1.028	0.999, 1.058	0.06	382	1.074	1.036, 1.113	<0.01	<0.01
2	557	1.038	1.004, 1.072	0.03	329	1.077	1.032, 1.124	<0.01	0.14
3	335	1.008	0.957, 1.062	0.77	197	1.104	1.043, 1.170	<0.01	<0.01
4	447	1.042	1.004, 1.081	0.03	268	1.071	1.021, 1.123	<0.01	0.17

Table 4 presents the stratified analysis of associations between maternal BMI and consultations and prescriptions for respiratory symptoms. Maternal BMI was only associated with wheezing illnesses among mothers with a history of atopy. The associations with prescriptions of inhaled corticosteroids or short-acting β2 agonists within children of an atopic mother were stronger compared to the children of non-atopic mothers and could not be explained by current weight and height or lung function (all p-values <0.01), while in non-atopic mothers current weight and height or lung function did explain the association.

Discussion

This is the first study that explored if neonatal lung function could be an intermediate factor in the association between maternal BMI and wheezing illnesses in the offspring. Our results showed that lung function could partly explain the association between maternal BMI and increased risk of wheezing in the offspring in their first year of life. Even at the age of 5 years, infant lung function could explain part of the association with consultations for wheezing illnesses. However, weight and height of the child at the age of 5 years seemed to play a greater role. Maternal BMI was also associated with increased number of prescriptions for short-acting β2 agonists and inhaled corticosteroids. It was remarkable that these associations, especially in children of atopic mothers, could not be explained by any of the potential confounders or intermediate factors. Whether disease status is better reflected by consultations or

prescriptions for respiratory illnesses is unknown. Probably, well-controlled asthmatic children have more prescriptions than consultations for wheezing illnesses. A hypothesis could be that children with a lower baseline lung function have symptoms of wheezing illnesses, which are more difficult to control with medication and therefore have more consultations at the general practitioner. Off all children with prescriptions of short-acting β2 agonists and consultations for wheezing illnesses, the children with more consultations than prescriptions had a higher resistance in infancy (7.07 versus 7.93, p-value <0.01), however this was not the case for prescriptions of inhaled corticosteroids.

Our findings of increased respiratory illness in the offspring of mothers with a higher maternal BMI are in line with previous literature.[5, 6, 9, 18, 19] Familial predisposition could be an effect modifier of the association, however the results of previous studies are inconsistent. Among adolescents the increased risk was seen only among those without a parental predisposition.[7] In our study we observed an increased risk of wheezing illnesses among children of mothers with a history of atopy, which is in line with other studies in childhood.[5, 18]

Our study provides further evidence that potential confounders could not explain the associations. Previous research has shown that intermediate factors, like pregnancy complications and postnatal growth, do not affect the association either.[5, 9] In our study we also adjusted for postnatal growth and current lung function at the age of 5 years, which confirmed these results (data not shown).

There are several strengths of this current study. Data of lung function, measured before the age of 2 months, was available in more than 2000 infants. The sample size of our study is large and data were collected in a standardized manner. Data of wheezing in the first year of life were collected on a daily basis and prescriptions and consultations of the general practitioner were derived from the GP electronic patient files. Most previous studies were based on parent-reported symptoms derived from a retrospective questionnaire, which increases the risk of recall bias and parental misclassification.[5, 6, 9, 18, 19] We obtained information of wheezing in the first year of life from daily questionnaires with parent-reported symptoms. We minimised the risk of misclassification by careful parental instruction. Due to the prospective data collection, recall bias in our study is of no (or minor) influence. Besides parent-reported symptoms, we were also able to study the association with data of consultations and prescriptions for respiratory illnesses derived from the electronic patient file of the general practitioner till the age of 5 year of the offspring.

Some limitations of the current study need consideration. First, selective follow up could affect the associations studied. Especially in the first year of life where a lot of effort of the parents was requested, selected children could have missing data. We imputed the data of the children with no complete follow-up in their first year of life and results were comparable. Second, weight and height of the parents were self-reported which could have introduced an underestimation of persons with a higher BMI. However, almost 1/3 of the parents participated in the UHP and in these parents weight and height were measured. A sensitivity analysis including only mothers with BMI calculated from actually measured weight and height resulted in similar results (results not shown).

As our study has shown that infant lung function could partially explain the associations in early life, it suggests that there could be a direct negative effect of factors, which are elevated in mothers with a higher maternal BMI on airways structure in the offspring. The exact mechanism needs to be elucidated, but as previous studies have shown that leptin plays a role in lung development, it would be interesting to further explore this. Off course, other adipokines, inflammatory or immunological factors could also play a role in this mechanism. Given the strong associations between maternal BMI and wheezing illnesses and the high prevalence of respiratory symptoms in childhood, it would be useful to gain more knowledge about the exact mechanism. A causal relationship could provide new targets for preventive interventions, as maternal obesity could be an important modifiable risk factor. Reducing weight in women of childbearing age could therefore improve health of both mother and child.

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Chapter 7

Relation between leptin and lung function in young healthy children

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Abstract

Background

Animal studies have shown that leptin plays a role in respiration and lung development. The role of leptin in the development of healthy human lungs is unknown. In this study we evaluated the relation between leptin and lung function in healthy children and we assessed whether this relation was modulated by the amount of adipose tissue.

Methods

Within the WHISTLER birth cohort 138 8-year-olds had successful spirometry and measurement of leptin in plasma. Linear regression analyses were performed with adjustments for age, sex, anthropometric measurements, including ultrasonographic measurement of intra-abdominal and subcutaneous adipose tissue, and potential confounders.

Results

Higher leptin was associated with lower lung function. The linear regression coefficient of the natural logarithm of leptin was -0.06 L/(ng/mL) (95% CI -0.11 to -0.01), which indicates that with a 10% increase in leptin, FEV1 decreased with 5.7 mL. Adjustment for weight attenuated the regression coefficient to -0.12 L/(ng/mL) (95% CI -0.17 to -0.08). Adjustment for adipose tissue and potential confounders barely changed the regression coefficient of leptin. Gender or weight were no effect modifiers (p-value interaction respectively 0.96 and 0.28).

Conclusion

In childhood, higher leptin is associated with lower lung function. This association is independent of the amount of adipose tissue.

Introduction

Leptin, a product of the obese (ob) gene, was discovered in 1994 as a hormone that plays a key role in regulating energy intake and expenditure.[1] Over the past years, other functions of this pleiotropic hormone have become of growing interest. Leptin is primarily produced by adipocytes and in lower amounts in other tissues. In the human lung, expression of leptin is described in bronchial epithelial cells and alveolar macrophages.[2, 3] Leptin receptors have a universal distribution, including the respiratory system. Receptors have been identified in the human bronchial and alveolar epithelial cells, bronchial smooth muscle cells, and bronchial submucosa.[4, 5] The presence of these receptors together with evidence of local leptin production supports the concept that leptin may play a role in the regulation of airway diameter, lung development and potentially the pathogenesis of respiratory diseases.[6] Recent studies have shown that leptin plays a role in lung development in rodents. Studies in ob/ob mice showed that leptin deficiency leads to impaired alveolar formation and lower lung volumes at birth. Postnatal treatment with daily intraperitoneal leptin injection in these mice increased the alveolar count and lung volumes.[7] A study in rats also showed that antenatal administration of leptin increased fetal lung weights, due to an increase in type II alveolar cells.[8] Another study in mice revealed that leptin is a regulator of bronchial diameter. Leptin caused bronchodilatation by signaling outside the hypothalamus in cholinergic neurons to eventually inhibit parasympathetic signaling through the M3 muscarinic receptor in airway smooth muscle cells.[9] Human studies mainly focus on the role of leptin in respiratory disease, like asthma and COPD.[10] Studies about the role of leptin in lung development have only been performed in animals and it is unknown if these findings could be extended to the development of healthy human lungs. According to these findings in animals, we hypothesized that during periods of development of the human lung, higher leptin is related with a more favourable lung function. In this study we evaluated the relation between plasma leptin levels and lung function in healthy young children and we assessed whether this relation was modulated by the amount of adipose tissue depots, the main source of leptin.

Methods

Setting and participants

Participants of this study were children from the Wheezing Illness Study Leidsche Rijn (WHISTLER), an ongoing population-based birth cohort on determinants of wheezing illnesses, initiated in 2001.[11] Healthy newborns in a new residential area near Utrecht City, Leidsche Rijn, were enrolled. Exclusion criteria were gestational age less than 36 weeks, major congenital abnormalities and neonatal respiratory disease. Currently, over 2800 children have been included. In 2007 this study was extended for cardiovascular research questions. All 5-year-olds were invited for follow-up measurements. In 2010, all children who participated in the 5-year measurements were invited for a second follow-up visit at the age of 8 years. Data on primary care visits were obtained from the general practitioner's electronic patient files.

This study is approved by the Medical Ethical Committee of the University Medical Center Utrecht. Written informed parental consent was obtained.

Neonatal visit and follow-up in infancy

Parents visited the clinic with their offspring between 3 and 8 weeks of age for lung function and blood pressure measurements. At this visit, parents were asked to fill in a questionnaire, including information on birth weight, birth length and gestational age.

Information about weight, height and feeding of the child during the first year of life was gathered by a monthly questionnaire. In the Netherlands, infants regularly visit child healthcare centers for standardized anthropometry. Anthropometrics are recorded in a personal file, which every child owns. Parents were asked to use this file to report the anthropometric measures.

Visit at the age of 8 years

Questionnaire

Information of the child and parents was gathered by a questionnaire.

Anthropometrics

Weight, height and circumferences of chest, waist and hip were measured. Measurements were performed with the participants wearing indoor clothes without shoes. Standing with the feet slightly apart chest, waist and hip circumference were measured in duplicate. Chest circumference was measured at the level of the nipples. Waist measurements were made halfway between the 10^{th} rib laterally and the most superior part of the anterior superior iliac crest and hip measurements halfway between the anterior superior iliac crest and the greater trochanter.

Ultrasound of adipose tissue

In addition, intra-abdominal and subcutaneous fat were measured using ultrasound according to a previously described method[12] with a Picus Pro system (Esaote, Italy), using a CA 421 convex transducer. For intra-abdominal fat, the distances between the posterior edge of the abdominal muscles and the lumbar spine were measured using electronic calipers. Distances were measured from three different angels: medial, left and right lateral, with the transducer placed longitudinally on a straight line drawn between the left and right midpoint of the lower rib and iliac crest. Measurements were performed at the end of a quiet expiration. The average distance was calculated from the three angles. Placing the probe transversely at the level of the umbilicus subcutaneous fat was measured with electronic calipers, from the external face of the rectus abdominis muscle (linea alba) to just below the skin. The measurement was repeated three times and the average was used for analysis. For all measurements, minimal pressure was applied to eliminate manual compression of tissue. Intraclass correlation coefficients (ICC) based on measurements by one observer in 10 subjects for intra-abdominal fat and, respectively 11 subjects for subcutaneous fat on 2 different occasions were 0.67 and 0.96, respectively. ICCs for subcutaneous

fat on the three measurements per child on the same occasion were 0.94, 0.94, and 0.97 for the three observers.[12] All measurements were performed by trained personnel.

Leptin measurement

A home-visit between 7:00 and 10:30 AM for venipuncture was scheduled after an overnight fast. A blood-sample was taken in a sodium-heparin tube, which was immediately placed on ice. The samples were centrifuged at 1450*g* (3000 rpm) at 4°C for 15 minutes. Within a maximum of four hours after the blood sample was taken, sodium-heparin plasma was stored at -80°C. A multiplex adipokine immunoassay, Luminex (Biorad, Munich, Germany) of which details have been described previously was used for determination of the concentration of different adipocytokines, including leptin.[13]

Lung function

Lung function was measured using a heated Lilly head pneumotachometer system (Viasys Healthcare, Hochberg, Germany) conform the latest American Thoracic Society (ATS)/ European Respiratory Society (ERS) statement for lung function measurements.[14] Measurements were corrected for body temperature, pressure and saturation. At least two reproducible flow-volume curves were obtained. The largest forced expiratory volume in 1 second (FEV1) was selected.

Covariates

Several exposures in childhood could influence both leptin level and lung function. Smoking is one of these potential confounders, although the effect of smoking on leptin level is not yet definitive.[15] The same applies to breastfeeding.[16, 17] In the analysis we used two variables for smoke exposure. The first one is smoke exposure of mother during pregnancy and the second one is current smoking of one of the parents. We divided the duration of breastfeeding in 4 categories: no breastfeeding, 0-3 month, 3-6 months and >6 months.

Postnatal growth the first three months of life is associated with reduced lung function at the age of 5 years[18] and increased amount of general and central adipose tissue[12]. It was defined as the weight gain rate of at least two measurements available in the first 3 months adjusted for the length gain rate. This method was previously described.[12]

During infections and inflammation leptin levels increase.[19] In our prospective cohort study we obtain General practitioner (GP) diagnosed infections using the International Classification of Primary Care (ICPC) codes from the GPs electronic files (Medicom, PharmaPartners, the Netherlands) from birth until the date of visit (at age 8 years). GP diagnosed infections were defined according to the infectious diseases subgroup of ICPC component seven. Per child the number of GP diagnosed infections from birth until the date of visit was summed, as measure of cumulative infection exposure. We developed 4 categories. The first group experienced 0-2 infections till the age at visit, the second group 3-5 infections, the third group 6-9 and the last group >10 infections till the age at visit.

Previous studies have provided evidence for a possible link between leptin and allergies.[20] We defined

an ever diagnosed allergy according to the following ICPC codes: allergic reaction (A12), allergic conjunctivitis (F71), asthma (R96), allergic rhinitis (R97), constitutional eczema (S87) and urticaria (S98).

Data analysis

Differences in subject characteristics between below and above median leptin levels were tested using independent T-test for continuous variables and χ^2 for frequencies.

The relation between leptin and lung function was assessed using linear regression analysis, with leptin as independent variable and lung function as dependent variable. Using multivariable regression models we adjusted for several possible confounders. Missing data on determinants were imputed using multiple imputations and the analysis was performed in 10 imputed data sets. The pooled results are expressed as linear regression coefficients with 95% confidence intervals (95% CI) and corresponding p-values. Due to increasing variance across the residuals (heteroscedasticity) of the linear regression model with leptin, we entered leptin in the model after natural log transformation.

It is known that females have already in childhood higher levels of leptin.[21] We assessed if sex could be an effect modifier of this association. Therefore, we stratified the data by sex and performed a linear regression analysis with adjustment for weight as described above. To test if modification by sex was statistically significant we added the product of sex and leptin as interaction term to the regression model. We performed the same analysis with weight instead of sex to test if weight could be an effect-modifier as well. We stratified the data in quartiles according to weight.

Statistical significance was considered reached at p-value <0.05. All analyses were performed with SPSS version 20.0 for windows (IBM, Armonk, New York, USA).

Results

Table 1 describes the baseline characteristics of the individuals by high versus low leptin levels. The children with lower leptin levels were older (8.3 (standard deviation (SD) 0.3) versus 8.0 (SD 0.5) years, p-value<0.01), had a lower weight (27.6 (SD 3.5) versus 28.6 kg (SD 5.3), p-value <0.01) and a smaller waist circumference (57.5 (SD 3.9) versus 59.3 cm (SD 5.8), p-value 0.03) than children with higher levels of leptin. There were fewer girls in the group with a lower level of leptin (66.6% versus 50.8%, p-value 0.06). No significant differences were observed in exposure to environmental factors.

Table 1. *Baseline characteristics*

	Low leptin (n=69)		High leptin (n=69)		
	N	**Mean**	**N**	**Mean**	**p-values**
Age (years)	69	8.3 (0.3)	69	8.0 (0.5)	<0.01
Height (cm)	69	132.8 (5.2)	69	132.1 (5.7)	0.50
Weight (kg)	69	27.6 (3.5)	69	28.6 (5.3)	<0.01
Gender (n, %)					
Female	35	51	46	67	0.06
Male	34	49	23	33	
Thoracic circumference	68	62.5 (3.3)	69	63.3 (5.4)	0.27
Waist circumference	68	57.5 (3.9)	69	59.3 (5.8)	0.03
Hip circumference	68	67.1 (4.3)	69	68.4 (6.1)	0.15
Growth (weight for height)	51	0.1 (1.0)	61	0.1 (0.9)	0.87
Intra-abdominal fat (cm)	69	36.3 (7.2)	69	37.9 (7.4)	0.21
Subcutaneous fat (mm)	69	8.1 (4.0)	68	9.1 (5.9)	0.28
Duration breastfeeding (n, %)					
None	17	25	18	26	0.45
0-3 months	32	46	29	42	
3-6 months	12	17	12	17	
>6 months	4	6	10	15	
Missing	4	6	0	0	
Smoke exposure pregnancy (n, %)					
No	40	58	49	71	0.14
Yes	28	41	20	29	
Missing	1	1	0	0	
Smoke exposure at age 8 (n, %)					
No	43	62	41	59	0.79
Yes	16	23	17	25	
Missing	10	14	11	16	
Allergy diagnosis (n, %)					
No	34	49	28	41	0.54
Yes	26	38	27	39	
Missing	9	13	14	20	
No. of infections (n, %)					
0-2	14	20	8	12	0.53
3-5	16	23	13	19	
6-9	14	20	18	26	
≥10	16	23	16	23	
Missing	9	13	14	20	

All values are means (and standard deviations) unless otherwise indicated.

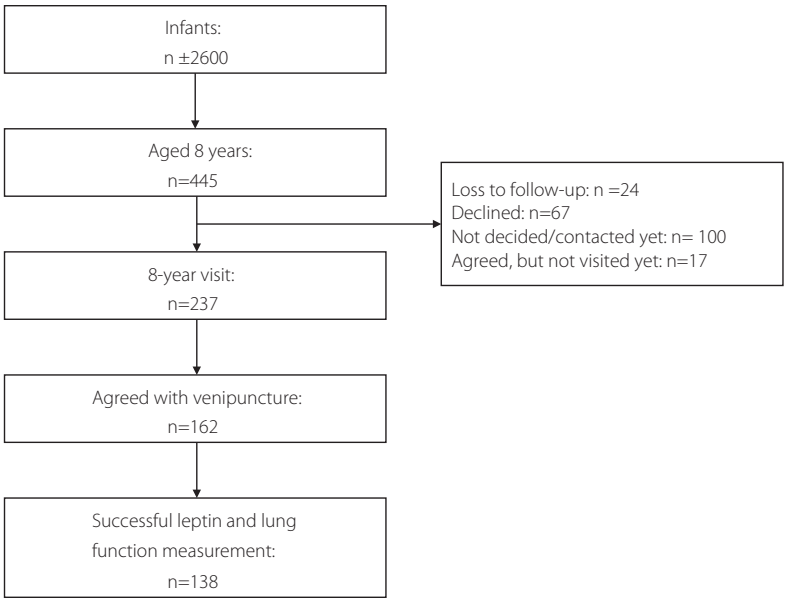

Figure 1. *Overview of the WHISTLER study population (July 2012)*

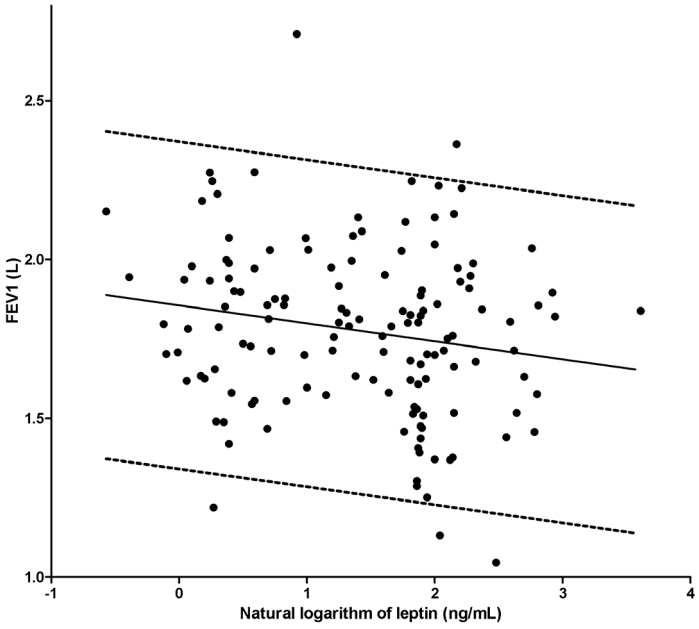

Figure 2. *FEV1 plotted against leptin levels*

Table 2 shows that without adjustments, leptin was negatively associated with FEV1. The linear regression coefficient of the natural logarithm of leptin was -0.06 L/(ng/mL) (95% CI -0.11 to -0.01), which indicates that with a 10% increase in leptin, FEV1 decreases with 5.7 mL (natural logarithm (1.1) *-0.06 L/(ng/mL)).

Adjustment for age and sex slightly attenuated the association to -0.04 L/(ng/mL) (95% CI -0.09 to 0.01) and -0.05 L/(ng/mL) (95% CI -0.10 to 0.00) respectively, while adjustment for anthropometrics increased the association. After adjustment for weight, a 10% increase in leptin was associated with an increase in FEV1 of 11.4 mL. The multivariable analysis barely changed the linear regression coefficient of leptin.

Table 2. *Relation between leptin and FEV1*

Adjustments	Linear regression coefficient	95% Confidence Interval	p-value
None	-0.06	-0.11, -0.01	0.03
Age	-0.04	-0.09, 0.01	0.12
Sex	-0.05	-0.10, 0.00	0.06
Height	-0.07	-0.11, -0.03	<0.01
Weight	-0.12	-0.17, -0.08	<0.01
Intra-abdominal fat	-0.07	-0.12, -0.02	0.01
Subcutaneous fat	-0.10	-0.15, -0.05	<0.01
Growth	-0.06	-0.07, -0.04	<0.01
Age, sex, weight, height	-0.10	-0.15, -0.06	<0.01
Breastfeeding, infections, allergies, smoking	-0.06	-0.12, 0.00	0.03

The regression analysis with FVC as dependent variable showed similar results, with almost exactly the same regression coefficients (supplementary table 1). The linear regression coefficients of the regression analysis with PEF were also similar, but in most models not statistically significant (supplementary table 2). The univariable model showed a linear regression coefficient of the natural logarithm of leptin of -0.06 L/(ng/mL) (95% CI -0.19 to 0.08). After adjustment for age, sex, weight and height the regression coefficient strengthened to -0.13 L/(ng/mL) (95% CI -0.27 to 0.02).

The relation between leptin and FEV1 did not differ between males or females. Adding an interaction term gender*leptin to the model showed no significant interaction (p for the interaction term 0.96). Indeed, within girls, the weight adjusted regression coefficient for the natural logarithm of leptin was -0.13 L/(ng/mL) (95% CI -0.18 to -0.07), within boys it was -0.12 L/(ng/mL) (95% CI -0.20 to -0.05).

Weight was also no effect-modifier of the relation between leptin and FEV1. Adding the interaction term weight*leptin to the model showed no significant interaction (p for interaction 0.28). Indeed, within increasing quartiles of weight, the weight-adjusted regression coefficients of the natural logarithm of leptin were -0.13 L/(ng/mL) (95% CI -0.19 to -0.07), -0,09 L/(ng/mL) (95% CI -0.19 to 0.02), -0.11 L/(ng/mL) (95% CI -0.19 to -0.03) and -0.14 L/(ng/mL) (95% CI -0.25 to -0.03) respectively.

Discussion

The present study shows that in childhood, higher leptin plasma concentration was associated with reduced lung function. This association was independent of the amount of adipose tissue, which is supposed to be the main source of circulating leptin.

Intra-abdominal or visceral adipose tissue is metabolically more active and produces more pro-inflammatory adipokines than subcutaneous adipose tissue, but the production of leptin is higher in subcutaneous adipose tissue.[22] In our study, adjustment for adipose tissue depots did not influence the association between leptin and lung function.

Previous studies of the role of leptin in lung development are all derived from animal models, which represents a major limitation in extrapolating the results to humans. The animal studies have shown that leptin is important for postnatal development of the lungs. Neonatal lambs receiving leptin intravenously showed that leptin concentration on the seventh day of life were positively correlated with lung weight.[23] Genetically obese, leptin deficient mice (ob/ob mice) exhibit significantly lower lung volume and lower alveolar surface area at 2 weeks of age, when compared to heterozygotes or control animals.[7] Other recent evidence in mice has shown that leptin favors bronchodilatation.[9] The parasympathetic nervous system signals in airway smooth muscle cells to cause bronchoconstriction. That study showed that leptin signaling outside the hypothalamus in cholinergic neurons inhibited the parasympathetic signaling in airway smooth muscle cells, which led to bronchodilatation. In obese mice bronchoconstriction occurred through leptin resistance.[9] However, there are differences in autonomic nervous system innervations and anatomy between mice and humans[24] and it is unknown if these findings can be extended to humans. From this perspective of animal studies one might expect that in healthy (non-obese) individuals higher leptin would lead to a more favourable lung function. However, we could not confirm this in our study concerning young children, in which we observed the opposite relationship. Differences could be due to differences in autonomic nervous system innervations. The latter study in mice reflects a model with either very low (lipodystrophic "fat-free" mice) or very high fat mass (diet-induced obese mice) in which leptin levels were either very low or high. However, this is not comparable with fat mass or leptin levels in our healthy group of children.

The direction of our findings is comparible with other studies performed in humans. A study performed in mainly asthmatic children showed a negative correlation between leptin and %predicted FEV1 and FEF25-75%.[25] Two large population-based studies assessed the same association and found similar results in adulthood.[26, 27] These studies were performed in non-obese adults and a group of African-Americans of which 86% of the participants were either overweight or obese. In our study, weight was not an effect-modifier in the association between leptin and lung function in childhood. As we have a healthy group of children with only a few overweight children, clinical leptin resistance is probably not present, although leptin resistance may gradually increase with increasing fat mass.

Other human studies exploring associations between leptin and the respiratory system mainly focused on clinically manifest respiratory disease. Several studies have suggested that leptin plays a role in the inflammatory pathogenesis of the co-morbidity of asthma and obesity. Four studies in children with

asthma and healthy control subjects were performed to further explore this hypothesis. Two studies showed increased levels of leptin in asthmatic children[28, 29], which might align with our findings, but two other studies showed no significant differences[25, 30]. A recent study performed in adult obese women showed that proinflammatory adipokines were increased in visceral adipose tissue of obese people with asthma and that visceral fat leptin was correlated with airway reactivity. However, it was not associated with airway inflammation, which suggests a direct effect of leptin on the airways.[31] Such findings may also concur with our findings.

A nested case-control study in rescue workers of 9/11 with exposure to WTC dust showed that leptin was an independent risk factor for greater susceptibility of FEV1 impairment. Leptin increased the odds of abnormal FEV1 by more than twofold after adjustment for BMI.[32] This longitudinal study supports the hypothesis that leptin has a direct effect on the airways, making reverse causation less likely. This is also confirmed in a study among obese adolescents with exercise-induced bronchospasm. After 1 year of interdisciplinary intervention promoting weight loss, leptin concentration reduction was a predictive factor for improvements in lung function.[33]

To our knowledge, this is the first study to demonstrate an association between lung function and leptin in healthy children. To appreciate our findings, some limitations need to be discussed. As this study has a cross-sectional design, only assumptions about causality of associations can be made. A longitudinal study could give us more insights into the development of the respiratory system and the role of leptin. We measured the children at the age of 8 years. It would be interesting to measure children from an earlier age onwards. However, the lung grows until late adolescence[34], and recent research showed that even alveolarization is ongoing throughout childhood and adolescence in humans.[35]

In conclusion, this is the first study to show an association between higher leptin plasma concentration and lower lung function, independent of adipose tissue in healthy children. The fact that we see such a strong association in healthy children supports the hypothesis that leptin has a functional role in the respiratory system.

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Supplementary table 1. *Relation between leptin and FVC*

Adjustments	Linear regression coefficient	95% Confidence Interval	p-value
None	-0.06	-0.12, 0.00	0.03
Age	-0.04	-0.10, 0.01	0.13
Sex	-0.05	-0.10, 0.01	0.12
Height	-0.07	-0.12, -0.03	<0.01
Weight	-0.14	-0.18, -0.09	<0.01
Intra-abdominal fat	-0.08	-0.13, -0.02	0.01
Subcutaneous fat	-0.10	-0.16, -0.05	<0.01
Growth	-0.06	-0.08, -0.05	<0.01
Age, sex, weight, height	-0.10	-0.15, -0.05	<0.01
Breastfeeding, infections, allergies, smoking	-0.06	-0.13, 0.00	0.04

***Supplementary table 2**. Relation between leptin and PEF*

Adjustments	Linear regression coefficient	95% Confidence Interval	p-value
None	-0.06	-0.19, 0.08	0.42
Age	-0.02	-0.16, 0.12	0.77
Sex	-0.04	-0.19, 0.10	0.53
Height	-0.08	-0.20, 0.05	0.23
Weight	-0.18	-0.31, -0.05	0.01
Intra-abdominal fat	-0.10	-0.24, 0.03	0.14
Subcutaneous fat	-0.13	-0.28, 0.01	0.07
Growth	-0.05	-0.09, -0.01	0.02
Age, sex, weight, height	-0.13	-0.27, 0.02	0.09
Breastfeeding, infections, allergies, smoking	-0.05	-0.20, 0.09	0.48

Part III

Methodological evaluation of new non-invasive devices to measure cardiovascular and respiratory development in childhood

Chapter 8

Feasibility and characteristics of arterial stiffness measurement in preschool children

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Submitted

Abstract

Introduction

Arterial stiffness is an important marker for the risk of developing cardiovascular diseases in adult life. Increasing evidence suggests that cardiovascular risk factors are already in childhood associated with increased arterial stiffness and other cardiovascular risk factors. Until now, little is known about measurements of arterial stiffness in preschool children. We aimed to assess the feasibility of arterial stiffness measurements and explore which determinants are related to arterial stiffness in preschool children.

Methods

We performed arterial stiffness measurements in three-year-old healthy infants, recruited from the Wheezing Illnesses Study Leidsche Rijn (WHISTLER). Using a noninvasive oscillometric device (Arteriograph) we measured aortic pulse wave velocity (PWVao) and augmentation index of the aorta (AIXao). Anthropometry was measured and other possible determinants were recorded using a questionnaire.

Results

In a total 168 infants, age ranged between 3.3 years and 4.1 years (mean age 3.7 years). In 100 subjects (59.5%) at least one valid measurement of arterial stiffness could be obtained. There were 89 infants who showed two or more successfully measurable curves, and 73 children in which at least three valid pulse wave curves were reported. Mean augmentation index of the aorta was 19.7 m/s (SD 7.0). Measurement of the augmentation index showed a significant inverse association with body height, with a regression coefficient of -0.78 (m/s)/cm (95% CI -1.13 – -0.42). Age, sex and (birth) weight showed no significant association with the augmentation index.

Conclusion

The feasibility of arterial stiffness measurements in preschool children is moderate. Height is the most important determinant of augmentation index in this age group.

Introduction

Arterial stiffness can be seen as an important marker for the risk of developing cardiovascular diseases in adult life. Arterial stiffness, which describes the loss of capability of an artery to react on pressure changes due to loss of elasticity, is influenced by several parameters. In adulthood, there is evidence for male sex, older age, increased weight and a lower height being the most important determinants of increased arterial stiffness.[1, 2] In childhood, low birth weight (small for gestational age), higher gestational age[3,4] and breastfeeding in the first year of life are associated with a higher arterial stiffness.[5] Furthermore, chronic inflammation could affect an increased arterial stiffness.[6]

There are several indicators for arterial stiffness, such as carotid-femoral pulse wave velocity (PWVao) and the augmentation index of the aorta (AIXao) and the brachial artery (AIXbr). While PWVao is used as a direct marker for arterial stiffness, augmentation index is used as a direct marker for wave reflection and an indirect marker for arterial stiffness.[7–9] In addition, central systolic blood pressure (SBPao) is an important parameter describing arterial stiffness and shows a strong correlation with an increased arterial stiffness when increased.[9] PWVao and AIX increase with the ageing process and both markers provide information about the status of the arterial system.[8]

Atherosclerosis, the leading cause of cardiovascular disease, starts to develop already in early life, however this does not directly cause symptoms in childhood.[5] Increasing evidence suggests that cardiovascular risk factors are already in childhood associated with increased arterial stiffness and other cardiovascular risk factors.[5, 10] These studies were mainly focused on children >5 years of age. Little is known about measurements of arterial stiffness in preschool children.

There are many different devices to measure arterial stiffness. However, many of them are not validated in childhood and not suitable to measure preschool children. The aim of this study was to describe the feasibility of measurements of arterial stiffness in preschool children, using the Arteriograph, a non-invasive oscillometric device. Secondly, we aimed to explore which determinants are related to arterial stiffness in preschool children.

Methods

Study population

We performed arterial stiffness measurements in three-year-old healthy infants, recruited from the Wheezing Illnesses Study Leidsche Rijn (WHISTLER), a population-based birth cohort study in Utrecht, the Netherlands.[11] For this study, healthy infants born in Leidsche Rijn, Utrecht, the Netherlands, were invited to participate. Children were excluded when gestational age was under 36 weeks, when they were born with congenital abnormalities or when they suffered from respiratory diseases. From December 2011 until April 2013 participants were invited for a follow-up visit at the age of three years. At this visit, the investigated subjects each underwent a physical examination, a lung resistance measurement using a MicroRint device (MicroMedical, VIASYS Healthcare, Kent, UK)[12] a transthoracic echocardiography and

arterial stiffness measurements. Study design of WHISTLER was described in detail earlier.[11] The pediatric medical ethics committee of the University Medical Centrum Utrecht, the Netherlands, approved the study. Written informed consent was obtained from the parents.

Questionnaire

We used questionnaires completed at the first neonatal visit, at approximately 4 weeks of age, to assess characteristics, including gender, age at visit, gestational age, birth weight and height, socio-economic status based on maternal education and exposure to environmental factors, such as tobacco smoke exposure during pregnancy. Information about the duration of breastfeeding was obtained from monthly taken questionnaires of the first year of life. Prior to the visit at the age of 3 years, the parents completed a new questionnaire containing information about current risk factors, like current smoke exposure of the child and health status of both the participant and the parents.

Anthropometric Measurement

During the visit, body weight of the children was measured wearing light clothing, using a standard electronic scale. Body height was measured without shoes, using an infant stadiometer. We measured the distance between the jugulum and the upper edge of the symphysis, measuring from sternal notch to the pubic bone, to be able to calculate the aortic Pulse Wave Velocity (PWVao).

Arterial Stiffness Measurement

The children were measured with the Arteriograph (Tensiomed, Budapest, Hungary), a non-invasive oscillometric device, which has been validated earlier in adults.[7–9] Measurements were taken in a supine position after 30 minutes of rest. The Arteriograph measures several parameters of arterial stiffness, such as blood pressure, PWVao and augmentation index.

Measuring the arterial stiffness is based on the physiological process of pulse waves. This pulse wave is measured in the brachial artery, with a brachial cuff.[13] As the cuff is pressurized, approximately 35 – 40 mmHg above the SBP, the brachial artery is completely occluded, so that oscillations can be recorded and pressure peaks can be detected. There are two systolic peaks that can be distinguished. First, there is the 'early' systolic pulse pressure wave (P1), which is formed by the left ventricle ejecting blood into the aorta. The 'late' systolic peak (P2) is due to the first pressure wave traveling along the aorta, being reflected at the site of the aortic bifurcation.[2, 13, 14] The return time (RT) of the systolic pressure wave, from the aortic root to the iliac bifurcation and back, is equal to the time lapses between the peaks of P1 and P2. PWVao can be calculated by halving this time and measuring the distance between the jugulum and the symphysis, which is comparable with the aortic length.[13] Augmentation index can be abstracted from the measured pulse pressure, and expresses the effect of the reflected wave.[15] The Arteriograph software gives an overall value of arterial stiffness, if all consecutive pulse waves can be analyzed. As pulse waves in early childhood are small and movement can distort the shape of a pulse wave, which could make evaluation of pulse waves impossible, we analyzed each cardiac cycle one by one using

the automatic Arteriograph software. A measurement was considered valid after a visual check and if the software was able to calculate a reliable value from the pressure wave curves. For every individual subject, we used the median of at least three valid curves.
Of randomly selected infants, we also measured the blood pressure with the Dinamap oscillometric device (Dinamap 1846 SX/P; GE Healthcare, Waukesha, Wisconsin).[16]

Statistical Analysis

The subject's characteristics are presented in percentages and means.. All variables were checked for normality of distribution. An intraclass correlation coefficient was used to determine the intrameasurement variability of average AIXbr, AIXao and PWVao based on 1 to 3 or more valid curve measurements. The correlation between the blood pressure measured with the Dinamap and the pressure measured with the Arteriograph was analyzed calculating Pearson's correlation coefficient.[17] Differences in characteristics of children with and without a valid measurement were tested using independent samples T-test for continuous variables or χ^2 for categorical variables. Univariable linear regression analysis was used to assess the associations of determinants based on previous literature, with the arterial stiffness parameters as dependent variables.
Statistical analysis was performed using SPSS Inc. version 21.0 for Windows (IBM, 2012, Chicago, Illinois, USA). A statistical significance was considered reached at p-value <0.05.

Results

We studied 168 healthy infants, ranging in age between 3.29 years and 4.11 years (mean age 3.72 years). An overview of the study population, showing the baseline characteristics including anthropometry, constitutional and environmental factors, is shown in table 1. Off these, 34 children were measured with the Dinamap device as well.

Blood pressure measurements with the Arteriograph succeeded in 136 subjects (81.0%). Of all children with no blood pressure measurement, 13 children refused to participate and 14 measurements could not be performed due to restlessness. We were not able to measure 3 children due to technical errors as a consequence of low battery and during the visit of 2 children measurements were prematurely stopped due to illness of the child.

Table 1. *Baseline characteristics (n=168)*

Subject characteristics		Mean	SD
Female sex (n, %)		93	55.4
Age at visit (years)		3.72	0.17
Gestational age (weeks)		39.2	1.4
Birth Weight (kg)		3.60	0.49
Birth Length (cm)		50.9	2.1
Length at visit (cm)		102.4	5.0
Weight at visit (kg)		17.2	2.0
Thoracic Circumference (cm)		54.0	2.7
Waist Circumference (cm)		52.9	3.5
Hip Circumference (cm)		51.3	3.5
Environmental factors			
Smoke exposure during pregnancy (n, %)	No	150	90.4
	Yes	16	9.6
Current smoke exposure (n, %)	No	164	98.8
	Yes	2	1.2
Breastfeeding (n, %)	None	30	17.9
	0 - 3 months	74	44.0
	3 - 6 months	43	35.6
	>6 months	21	12.5
Maternal education level (n, %)	Low/Moderate	36	25.2
	High*	107	74.8

*Values are means and standard deviations (SD) unless otherwise indicated. * Higher vocational or university education.*

In 100 subjects (59.5%) at least one valid measurement of arterial stiffness could be obtained. There were 89 infants who showed two or more successfully measurable curves, and 73 children in whom at least three valid pulse wave curves were reported. Table 2 shows an overview of the arterial stiffness measurements. Our study showed a mean augmentation index of the abdominal aorta of 19.7 m/s (SD 7.0) and aortic PWV of 5.56 m/s (SD 0.77) in children with ≥3 valid pulse waves. For every individual, we calculated the intraclass correlation coefficient (ICC) to determine the intrameasurement variability. The ICC for PWVao and AIXao for three or more occlusions were 0.87 and 0.72 respectively.

The mean value of systolic blood pressure measured by the Dinamap device was 95 mmHg (SD 6), where diastolic blood pressure measured showed a mean value of 58 mmHg (SD 6). In the same 34 children, systolic blood pressure measured by the Arteriograph showed a mean value of 94 mmHg (SD 7), with a diastolic blood pressure mean value of 50 mmHg (SD 5). Pearson's correlation coefficients of blood pressures measured using the Arteriograph and the Dinamap were 0.56 for systolic and 0.69 for diastolic blood pressure respectively.

The differences in child characteristics between individuals with and without a valid measurement (≥3 valid pulse wave analysis) are shown in table 3, which showed no significant differences.

Table 2. *Arterial stiffness parameters*

	Mean	SD
Arteriograph (n = 73)		
AIXbr (m/s)	-35.3	13.3
AIXao (m/s)	19.7	7.0
PWVao (m/s)	5.6	0.8
SBPao (mmHg)	86.7	6.9
PPao (mmHg)	36.2	4.3
MAP (mmHg)	65	5
Systolic Blood Pressure (mmHg)	94	7
Diastolic Blood Pressure (mmHg)	50	5
ED (ms)	275.8	17.7
RT (ms)	131.8	14.9
Heart Rate (1/min)	94	11
Dinamap (n = 34)		
Systolic Blood Pressure (mmHg)	97	6
Diastolic Blood Pressure (mmHg)	57	5
Heart Rate (1/min)	95	9

AIXbr: Augmentation Index of the brachial artery, AIXao: Augmentation Index of the aorta, PWVao: aortic Pulse Wave Velocity, SBPao: Systolic Blood Pressure of the aorta, PPao: aortic Pulse Pressure, MAP: Mean Arterial Pressure, ED: Ejection Duration, RT: Return Time. Values are means and standard deviations.

Table 3. *Differences in characteristics of children with and without a valid measurement*

		Not valid		Valid		
Subject characteristics		**Mean**	**SD**	**Mean**	**SD**	**p-value**
Female sex (n, %)		56.0	58.9	37	50.7	0.29
Age at visit (years)		3.70	0.17	3.74	0.16	0.22
Length at visit (cm)		101.9	4.8	102.6	4.1	0.34
Weight at visit (kg)		17.1	2.6	17.3	1.8	0.50
Smoke exposure during pregnancy (n, %)	No	83	87.4	67	94.4	0.13
	Yes	12	12.6	4	5.6	
Breastfeeding (n, %)	None	17	17.9	13	17.8	0.67
	0 - 3 months	44	46.3	30	41.1	
	3 - 6 months	21	22.1	22	30.1	
	>6 months	13	13.7	8	11.0	
Maternal education level (n, %)	Low/Moderate	21	25.0	15	25.4	0.95
	High*	63	75.0	44	74.6	

*Values are means and standard deviations (SD) unless otherwise indicated. * Higher vocational or university education.*

Table 4 shows the influence of several subject characteristics on augmentation index of the aorta and aortic pulse wave velocity. Although the direction of the regression coefficients was conform earlier findings,[2] none of the selected characteristics were significantly associated with pulse wave velocity. Measurement of the augmentation index of the aorta showed a significant inverse association with body height, with a regression coefficient of -0.78 (m/s)/cm (95% CI -1.13 – -0.42). Age, sex, birth weight and current weight showed no significant association with the augmentation index.

Table 4. *Association between arterial stiffness en child characteristics in early life*

	AIXao (n = 73)			PWVao (n = 73)		
	Linear regression coefficient	**95% CI**	**p-value**	**Linear regression coefficient**	**95% CI**	**p-value**
Female sex	0.60	-2.68 – 3.87	0.72	0.25	-0.11 – 0.61	0.17
Age (years)	-5.73	-15.77 – 4.31	0.26	0.06	-1.07 – 1.18	0.92
Weight at visit (kg)	-0.75	-1.64 – 0.15	0.10	0.03	-0.07 – 0.13	0.57
Length at visit (cm)	-0.78	-1.13 – -0.42	<0.01	0.01	-0.03 – 0.06	0.53
Birth weight (kg)	1.53	-2.24 – 5.30	0.42	0.13	-0.29 – 0.55	0.53

CI: confidence interval.

Discussion

This is the first study in which feasibility of arterial stiffness measurement was assessed using the Arteriograph in a large group of preschool children. We were able to measure arterial stiffness (with at least 3 valid curve waves) in almost half of our study population.

One previous study already determined reference values in this age group, but they only studied a small group of preschool children. In this study, only stratification for sex and age was applied but associations of child characteristics and arterial stiffness was not studied. The mean pulse wave velocity and augmentation index of the aorta of our 3-year-olds are in accordance with this population.[2] Feasibility of these arterial stiffness measurements was not assessed previously in preschool children.[2]

In our study, only height was significantly associated with the aortic augmentation index. Other studies in childhood, using different devices found also evidence for age and weight as determinants of arterial stiffness.[1, 2, 5] Although the determinants were not significantly associated with pulse wave velocity or the augmentation index in our study, the trends of the regression coefficients were comparable to these previous findings. The age range of our study population was small, which might explain these differences in statistical significance. We saw no clear trend of the association with sex, while previous studies found evidence for increased arterial stiffness in males.[1, 2] As previous studies were performed in older age groups, sex differences probably develop at an older age. Influences of hormonal puberty-related changes could play a role in these differences.[5, 18, 19]

Our most important finding is that we were able to determine the feasibility of the Arteriograph device in a relatively large group of healthy three-year-old children. Many devices are not suitable to measure arterial stiffness in preschool children. Performance of an arterial stiffness measurement using the Arteriograph was relatively quick (a few minutes) and children experience only a little discomfort, comparable with a blood pressure measurement. To determine the intrameasurement variability we calculated the intraclass correlation coefficient, which showed a good correlation with an ICC for PWVao and AIXao for three or more occlusions per child of 0.87 and 0.72, respectively. We also calculated the correlation between the blood pressure measurements by using the Arteriograph en the Dinamap device, using a Pearson correlation coefficient. In our homogeneous study population with a low variability in age, weight and height, the values of these correlation coefficients can be considered strong.

A limitation of our study is that we performed the arterial stiffness measurements during a study visit with other measurements as well. The investigated subjects underwent a physical examination (length and weight), a lung function measurement and a transthoracic echocardiography before arterial stiffness measurements were obtained. However, this does reflects current practice and according to the manual of Arteriograph, a rest of at least 10 minutes is necessary before performance of the measurement.

Even though these are all completely non-invasive examination methods, there is a chance of dropout because of fatigue or restlessness of the children. As we were able to obtain at least three valid measurements per child in almost half of the study group, this number will probably increase in a study without performance of the other measurements.

The process of atherosclerosis starts in the young and increasing evidence suggests that early life adverse exposures could lead to structural changes in the vascular system. Therefore, it is important to gain more knowledge about the vascular system in early childhood. In our study, we only measured the arterial stiffness measurements at the three-year visit. It would be recommendable to perform similar measurements in follow-up visits later in life, so that tracking of the arterial stiffness can help us to display alterations in risk factors and arterial stiffness parameters.

With this study, we were able to determine the feasibility of arterial stiffness measurements in preschool children. Height is the most important determinant of augmentation index in this age group.

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Chapter 9

Nocturnal wheeze measurement in preschool children

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Abstract

Background

Wheezing is a very common symptom in preschool children. Nocturnal wheezing is present in many asthmatic patients, due to enhanced airflow limitation overnight. We assessed the prevalence of nocturnal wheezing in young children and correlated this with respiratory system resistance and history of wheezing symptoms.

Methods

Using a continuous overnight recording of respiratory sounds we analyzed wheeze rate (ratio between wheezing time and recorded breathing time), oxygen saturation and heart rate during one night in 59 3-year-old children of an ongoing birth cohort study, the WHISTLER-project. We associated the nocturnal measurements with the patient's history of wheezing symptoms and with measurement of respiratory system resistance (Rint).

Results

Analysis of wheeze rate was successful in 44 children. The overall wheeze rate of these children was low, with the highest wheeze rate of 0.63% measured by the tracheal sensor during expiration. In total, 21/44 children had a wheeze rate of ≥5% during at least one minute. There was no statistically significant difference in wheeze rate between the children with and without a history of wheezing. The wheeze rate of the tracheal sensor had a significant correlation with Rint (correlation coefficients of inspiration and expiration: 0.308 and 0.382, p-values 0.05 and 0.01, respectively).

Conclusion

Overall, the wheeze rate in young children is low, but seems to increase over night-time. Almost 50% of the children have sporadic wheeze during the night. Although higher nocturnal wheeze rates are related to increased respiratory system resistance, it is not related to clinical wheezing symptoms.

Introduction

Wheezing is a symptom which is highly prevalent in the first years of life.[1,2] It is often a transient condition and related to early life reduced small airway caliber and increased airway resistance.[3]

Worsening of symptoms including wheezing at night is a common complaint affecting more than two-thirds of asthma patients.[4,5] It contributes to increased asthma related morbidity and mortality and especially in childhood it has physical and developmental consequences.[6,7] Nocturnal symptoms in young children are often difficult to diagnose, because patients with these symptoms often do not wake up until severe obstruction of the airways occurs. Using an automatic wheeze detection device, Bentur et al. found an unsuspected high level of nocturnal wheezing in a group of untreated asthmatic children, including those with mild asthma and normal spirometry.[8]

An important factor contributing to nocturnal asthma is the increased airway resistance overnight due to changes in respiratory function related to sleep, as well as nonsleep-related effects, such as processes under circadian regulation.[9] It is unknown how common nocturnal wheezing is in asymptomatic preschool children and if it correlates with other clinical indices. Especially in young children who can not perform spirometry, objective measurement of nocturnal wheeze activity and response to treatment could be clinically useful.

In this study we aimed to study the prevalence of nocturnal wheezing in young healthy and asymptomatic asthmatic children and correlate this with actual respiratory system resistance and history of wheezing symptoms.

Methods

Setting and participants

The children of this study are participants of the ongoing Wheezing Illnesses Study Leidsche Rijn (WHISTLER). Design and rationale of the WHISTLER study have been described elsewhere.[10] Participants of the WHISTLER study are invited for a first visit to our outpatient clinic before the age of 2 months. Exclusion criteria are gestational age <36 weeks, neonatal respiratory disease and congenital abnormalities.

For the present study, as from December 2011, all children of the WHISTLER study who were 3 years old were re-invited to visit the Wilhelmina Children's Hospital. If children suffered a respiratory infection during the planned visit we rescheduled the appointment. The study has been approved by the Medical Ethics Committee of the University Medical Centre Utrecht and conformed to the standards set by the declaration of Helsinki. Written informed consent was obtained from all parents of the participants.

Measurements

Information of the participants was gathered by a health questionnaire. During the visit in the hospital we measured weight using a standard electronic scale and height using a stadiometer. We measured interrupter resistance using the MicroRint (Micro Medical Limited, Kent, UK), according to standardized methods.[11] Median Rint was calculated from at least 5 acceptable interruptions within children.

Nocturnal measurements

The nocturnal measurement of respiratory sounds was conducted during the night after the hospital visit by the parents at home. Recording and analysis of respiratory sounds were conducted according to standardized methods using the Pulmotrack 2020/3020 with WHolter (KarmelSonix).[8,12] This is an ambulatory wheeze and cough recorder that stores continuous information from two phonopneumography piezoelectric sensors with a frequency range of 80-2400 Hz, a pneumogram belt and an ambient microphone. Using adhesive foam pads, the sensors were attached to the skin above the manubrium and at the right mid axillary line above the 5th intercostal space. The pneumogram belt was fitted above the lowermost ribs. The pneumogram belt contains tension sensors and differentiates between inspiratory and expiratory wheezes and determines the respiratory rate and the Inspiration/Expiration ratio. At the same time, oxygen saturation and heart rate were measured (Wrist pulse oximeter MD300 W) with a sensor attached to the toe.

During hospital visits parents were instructed about how to attach the devices and start the recordings and were given a leaflet with this information in writing. Recording ran continuously during the night and was started when the child went to bed, or when the parents went to bed, if they preferred to start the measurement when the child was asleep. If the child used asthma medication on a regular basis, this treatment was continued as usual.

The nocturnal recording was automatically analyzed by the Pulmotrack software. The definition of wheezing used by this software is in accordance with the guidelines of the European Task Force report to standardize definitions and terminologies used in computerized lung sounds analysis.[12] Wheezing was defined as continuous adventitious breathing sounds having a musical character and lasting longer than 100 milliseconds. The Pulmotrack system determines the wheeze rate per minute. Wheeze rate is the ratio between overall wheezing time and overall recorded breathing time.

Disconnection of the wires with the sensors due to movement of the child is not visible in the overall output of the Pulmotrack Software, but only after selection of a certain recorded minute. During a visual and auditive check of the recorded data, periods of wire disconnection were discarded. Due to the frequent occurrence of disconnection, we selected from each nocturnal measurement three periods of 30 minutes of sleep with a recording without signs of disconnection. Sleep was defined as no vocal sounds on the ambient microphone and a stable respiration rate and inspiration: expiration ratio. Wheeze rate was determined during the first 30 minutes of sleep in the three time periods, respectively 8.00 p.m.-11.00 p.m., 11.00 p.m.-2.00 a.m. and 2.00 a.m.-5.00 a.m, with at least 60 minutes between the first and the second period. Oxygen saturation and heart rate data were expressed as the mean per minute.

Data analysis

Central estimators and variance measures to describe general characteristics were calculated. All variables were checked for normality of distribution. Wilcoxon Signed Rank tests were used to compare the wheeze rates in the different time periods. Children with a complete nocturnal recording (successful

recordings in the 3 time periods) were compared with children with a successful recording in only 1 or 2 time periods using the Mann-Whitney U test. This test was also used to compare the groups of children with wheezing symptoms in the past and the use of asthma medications. For this analysis the mean wheeze rate of the three periods was used. Spearman Rank correlation coefficients were used to examine the relation between wheezing and airway resistance. Independent T-tests were used to compare the airway resistance in the groups of children with and without wheezing symptoms in the past, the use of asthma medication and with at least 1 minute wheeze rate >5% in our nocturnal measurement. P-values <0.05 were considered statistically significant. All statistical analyses were performed with SPSS version 20.0.

Results

Table 1 describes the baseline characteristics of 59 included children. In 15 children no valid recording could be obtained (7 not started correctly by the parents, 4 children refused recordings, 4 children with too short recordings (less than 30 minutes) due to disconnection of the wires). In the first period (between 8.00 -11.00 p.m.) 41 children had a successful recording, in the second period (between 11.00 p.m. - 2.00 a.m.) 33 children had a successful recording and in the last period (2.00 -5.00 a.m.) 18 children had a successful recording. In total, 16 out of 59 children had a successful recording in each time period.

Table 1. *Baseline characteristics of the study (n=59)*

		Total (n=59)	**Successful measurement (n=44)**
Sex (male/female)		27/32	17/27
Age (years)		3.8 (0.2)	3.8 (0.2)
Length (cm)		103.1 (5.0)	103.0 (5.1)
Weight (kg)		17.4 (2.1)	17.6 (2.1)
Interrupter Resistance (kPa/L/s)		0.88 (0.2)	0.91 (0.2)
Ever wheezing? yes/no (n)		19/40	15/29
Wheezing in the last 12 months diagnosed by the parents (n)		9/50	8/36
Wake-up because of wheezing in the last 12 months? (n)	Never	52	38
	<1 night/week	5	4
	>1 night/week	1	1
	unknown	1	1
Use of asthma medication in the last 3 months? yes/no (n)		6/53	4/40

Data are presented as mean (standard deviation) unless otherwise indicated.

The Wholter and Pulsoximetry parameters per period are summarized in table 2. All different wheeze rates (inspiration and expiration, tracheal and chest sensors) increased during the night, however this was not statistically significant except for the chest wall sensor during expiration. The increase in median

wheeze rate of this sensor comparing period 1 and 2 (n=30) and comparing period 1 and 3 (n=16) was statistically significant (p-values respectively 0.047 and 0.015).

Table 2. *Wholter and pulsoximetry parameters for the three time periods*

	Period 1 8 pm - 11 pm (n=41)		**Period 2 11 pm - 2 am (n= 33)**		**Period 3 2 am – 5 am (n= 18)**	
	Median	**IQR**	**Median**	**IQR**	**Median**	**ICR**
Tracheal Sensor (Inspiration) Wz%	0.18	0.12-0.27	0.22	0.10-0.35	0.24	0.14-0.34
Chest Wall Sensor (Inspiration) Wz%	0.31	0.07-0.50	0.35	0.20-0.63	0.49	0.31-0.77
Tracheal Sensor (Expiration) Wz%	0.21	0.11-0.66	0.43	0.16-0.80	0.63	0.44-0.70
Chest Wall Sensor (Expiration) Wz%	0.20	0.08-0.38	0.33*	0.16-0.59	0.52#	0.18-1.18
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Respiration rate/min	22.5	3.9	21.1	6.7	24.1	12.0
Inspiration: Expiration Ratio	0.68	0.21	0.68	0.30	0.62	0.17
	Period 1 (n=30)		**Period 2 (n= 24)**		**Period 3 (n= 9)**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Saturation (%)	97.9	1.3	97.8	1.6	98.8	2.6
Heart rate (rate/min)	89.3	11.1	84.2	6.7	89.4	24.4

*Data are presented as medians with interquartile range or means with standard deviation as indicated. *The increase in median Wheeze Rate comparing period 1 and 2 (n=30) was statistically significant, p-value 0.047. #The increase in median Wheeze Rate comparing period 1 and 3 (n=16) was statistically significant, p-value 0.015.*

Previous studies described that a wheeze rate <5% is considered not clinically significant.[8, 13, 14] Table 3 shows the number of children with wheeze rate ≥5% during at least one minute in one of the three periods per sensor during inspiration and expiration. In total, 21 children had a wheeze rate of ≥5% with one of the sensors (during inspiration or expiration) during 1 minute in one of the three periods. Nine children had a wheeze rate of ≥5% during 1 minute during inspiration and 18 children during expiration**.** Six children wheezed in both inspiration and expiration. More wheeze was detected during expiration and in the tracheal sensor.

The wheeze rates (mean of the different time periods) between the children with a successful recording in all three time periods was higher for the Chest Wall sensor inspiration and expiration (respectively wheeze rates 0.24 vs. 0.34, p-value 0.01 and 0.37 vs. 0.50, p-value 0.04) and for the Tracheal sensor inspiration and expiration (respectively wheeze rates 0.24 vs. 0.34, p-value 0.04 and 0.67 vs. 0.77, p-value 0.08) compared to the children with only 1 or 2 successful recordings. When comparing the different time periods with each other there was still a trend of higher wheeze rates in the children with a complete

nocturnal measurement, but this was not significant except for the Chest Wall Sensor Inspiratory and Expiratory in the second period 2 (p-values 0.05 and 0.03 respectively).

Table 3. *Number of children with ≥5% Wheeze Rate during at least one minute*

	Period 1 8 pm – 11 pm (n=41)	Period 2 11pm–2 am (n=33)	Period 3 2 am-5 am (n=18)
Tracheal Sensor Inspiration	3 (7.3)	4 (12.1)	1 (5.6)
Chest Wall Sensor Inspiration	1 (2.4)	2 (6.1)	1 (5.6)
Tracheal Sensor Expiration	8 (19.5)	6 (18.1)	4 (22.2)
Chest Wall Sensor Expiration	2 (4.8)	2 (6.1)	2 (4.2)

Data are presented as number (%) of children

Comparisons of the wheeze rates (mean of the three periods) between the groups with and without a history of wheeze and between the groups with and without the use of asthma medication in the past 3 months are shown in table 4. There was no statistically significant difference in wheeze rate between the children with and without a history of wheeze. Four children with a successful nocturnal recording used asthma medication in the past 3 months because of a diagnosis of asthma. They all used a short acting beta agonist and three out of four children used inhaled corticosteroids as well. There wheeze rates tended to be lower compared to non-treated children. This was only statistically significant (p-value 0.002) for the tracheal sensor during inspiration.

Table 4. *Comparison of Wheeze Rates between the groups with and without a history of wheeze and asthma medication in the past 3 months.*

	Ever wheezing			Asthma medication		
	Yes (n=15)	No (n=29)	p-value	Yes (n=4)	No (n=40)	p-value
Tracheal Sensor Inspiratory Wz%	0.30 (0.13-0.59)	0.18 (0.14-0.26)	0.293	0.06 (0.05-0.10)	0.20 (0.14-0.48)	0.002
Chest Wall Sensor Inspiratory Wz%	0.48 (0.19-0.74)	0.28 (0.13-0.48)	0.970	0.30 (0.15-0.54)	0.34 (0.14-0.59)	0.738
Tracheal Sensor Expiratory Wz%	0.45 (0.24-0.74)	0.50 (0.17-0.86)	0.162	0.21 (0.07-0.39)	0.53 (0.20-0.92)	0.109
Chest Wall Sensor Expiratory Wz%	0.45 (0.12-0.92)	0.22 (0.14-0.46)	0.134	0.19 (0.09-0.41)	0.29 (0.14-0.69)	0.441

Values are expressed as medians (interquartile range). Wz% is the mean of the three periods.

The wheeze rate of the tracheal sensor during inspiration and expiration (mean of the three time periods) had a significant correlation with the actual measurement of respiratory system resistance, Rint (correlation coefficients and p-values respectively 0.308 (p-value 0.05) and 0.382 (p-value 0.01)). There was no significant correlation between the wheeze rates of the different time periods of the Chest Wall sensor. When comparing the children without or with at least 1 minute wheeze rate ≥5% in one of the

three time periods, there was a trend towards a higher resistance in the group of children with at least 1 minute wheeze rate ≥5% (respectively 0.87 kPa/L/s versus 0.96 kPa/L/s, p-value 0.15).
The mean airway resistance in the group of children with asthma medication was not significantly different compared to the group with no asthma medication (mean airway resistance respectively 0.93 kPa/L/s versus 0.88 kPa/L/s, p-value 0.63), which was the same for children with or without wheeze symptoms in the past (mean airway resistance respectively 0.89 kPa/L/s versus 0.88 kPa/L/s, p-value 0.81).

Discussion

Overall, children from a general population birth cohort have a low prevalence of wheezing, which seems to increase during the night. However, in almost 50% of the children occasionally wheeze rates above 5% were found. There was no correlation with a history of wheeze, but wheeze rates correlated significantly with actual measurements of respiratory system resistance.
Objective parameters of airway disease are almost lacking in young children, especially in preschool children who are too young to perform spirometry. As far as we know, these nocturnal measurements of wheezing sounds were never performed before in children of this age. We found wheeze rates above 5% in almost 50% of the children, which is considered to be significant by other authors.[8, 13, 14] We only measured the children for a maximum of 90 minutes (3 periods of 30 minutes), so probably the true number of is even higher.
It was remarkable that we saw higher wheeze rates in the children with a successful recording in all three time periods. Partly, it could be explained by the fact that these children have more often a successful recording in the third period. When we compared the different time periods with each other we saw a trend of higher wheeze rates in these children as well. The reason for this trend is unknown.
In the group of children with no asthma medication we saw a trend towards more wheezing in this group compared to the group of children who used asthma medication in the past 3 months. Although the number of children with asthma medication was low (4 children), it was significant for the tracheal sensor during inspiration. This might point to a decrease of airflow limitation in these children or an enhanced airflow in the children with asthma medication which could be a result of the use of beta agonist during the measurement. The clinical relevance of this finding is unknown. Due to the small number of children with asthma medication it could also be false positive finding.
We saw no difference in airway resistance between the children with and without asthma medication or with and without wheeze symptoms in the past. A possible explanation is that wheeze analysis is performed during the night and measurement of airway resistance in the afternoon. Wheeze rates correlated significantly with Rint measurements. These findings suggest that analysis of nocturnal wheezing sounds could be a useful tool to substantiate.
A study of Prodhan et al. compared the inter-rater agreement about wheeze detection in PICU patients using the Pulmotrack.[15] They compared auscultation findings of respiratory therapists, nurses, physicians

and the Pulmotrack to those of an expert panel and conclude that the Pulmotrack was better than the staff in detecting wheeze (sensitivity 0.75% and specificity 0.76%).

Continuous acoustic monitoring during one night at home appears difficult to perform using the Pulmotrack. We had a high number of children in whom the performance of the measurement failed. We instructed the parents how to start the device and gave them an information letter. Unfortunately it was only possible to start the Pulmotrack once. If the parents accidentally pressed the start button the second time the device switched off and it was not possible for the parents (without the software of Pulmotrack) to restart it. We had only 16 out of 59 children with a good recording in three consecutive time periods over night. Young children are often physically active during sleep, which results in disconnection of the wires. Analysis is done by the Pulmotrack software, but interpretation of the results is time-consuming. Artifacts due to disconnection of the wires can only be discarded after visual and auditive control of the complete recordings.

As far as we know, only three articles are published about nocturnal measurements in small numbers of children using the same Pulmotrack device.[8, 13, 14] The patients in all three studies were older (all >6 years old) and in at least two of these studies measurements were performed by a dedicated technician and not by parents. Although Bentur et al. describe that 2 of the 14 eligible patients were rejected due to technical reasons (failure to fulfill the protocol requirements)[13], no other difficulties with the performance of the measurement were described in these papers. In 12 asthmatic patients Bentur et al. found no significant relationship between wheeze rates and subjective complaints of nocturnal asthma. This is similar for children with a history of wheezing symptoms in our present study. Another study concluded that children with asthma have a considerable amount of wheeze during the night that is episodic in nature and poorly related to conventional measures of lung function, subjective symptoms or current estimates of control.[14] This is in accordance with our study in asymptomatic young children. The occurrence of wheezing in these children was episodic as well. A potential limitation of our study in young children is that only relatively short periods of sleep could be observed during only one night in asymptomatic children. Maybe therefore, the mean wheeze rates were very low. Technical difficulties hamper prolonged recordings in a substantial number of young children. Another limitation of this measurement might be that the analysis of wheezing is based on only two sensors placed on the manubrium and chest wall. Wheezing sounds could have been missed from other places in the lungs.

We conclude that wheeze can occur in young healthy and clinically asymptomatic asthmatic children, which increases over night-time and is associated with respiratory system resistance. However, the use of the Pulmotrack is rather complicated because of technical inconveniences, which precludes extensive studies using this method at this stage.

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Chapter 10

Characteristics of Southeast Asian infant lung function

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Submitted

Abstract

Background

Infant lung function in early life can predict respiratory diseases and later lung development. Due to technical challenges, studies on infant lung function were mostly done in Western countries. There is limited information on infant lung function in other parts of the world. We aimed to identify determinants of the lung function distribution in healthy Southeast Asian infants and to compare these with a Western population.

Methods

We performed a cross-sectional study in a mother and child hospital in Jakarta, from June to October 2012. Using the automated single occlusion technique (SOT) we measured lung function in 124 infants (aged 1-10 months). Anthropometry was measured and other possible determinants were recorded using a questionnaire. The data of compliance and resistance of the respiratory system were compared with data from the Wheezing Illnesses Study Leidsche Rijn (WHISTLER) in the Netherlands.

Results

Technically acceptable lung function measurements were obtained in 96 children (77.4% of all eligible subjects). With increasing age, weight and length, compliance increased and resistance decreased. With each kg increase in weight compliance increased with 12.70 mL/kPa (95% CI 9.44 to 15.95) and Rrs decreased with -0.47 kPa/L/s (95% CI -0.63 to -0.30). The increase and decrease were similar in the WHISTLER population (p-values interaction term of Crs and Rrs respectively 0.753 and 0.967). Children living in more polluted areas had a lower compliance (linear regression coefficients adjusted for weight -12.61 mL/kPa, 95% CI -22.84 to -2.38).

Conclusion

Our results suggest that besides age, weight and height, air pollution is an important determinant of infant lung function in Southeast Asian children.

Introduction

Lung function in early life can be used as a predictor of subsequent respiratory diseases and lung development. Reduced or diminished lung function in early life is associated with recurrent wheeze and asthma in later life.[1–3]

Infant lung function is probably influenced by both genetic and environmental factors,[4] but the number of studies on the influencing factors of healthy infant lung function is limited. There is evidence of several early life influences such as birth weight, gestational age and maternal smoking on lung function.[5,6] So far, studies have mainly been performed in Western countries, while determinants of infant lung function might be different between Western and non-Western countries.

Measuring lung function in infants is not easy and requires sophisticated methods. One method to measure infant lung function is the single occlusion technique (SOT).[7,8] This non-invasive method determines compliance (Crs), resistance (Rrs) and the expiratory time constant (Trs) of the respiratory system in spontaneously breathing infants during natural sleep. It has been used in a large birth cohort study in healthy infants called WHISTLER (Wheezing Illnesses Study Leidsche Rijn).[9] Guidelines regarding equipment,[10] signal processing, data handling,[11] control and acceptance criteria[8] have been published by the European Respiratory Society (ERS)/American Thoracic Society (ATS) Task Force on standards for infant respiratory function testing. Nowadays, an affordable, portable and automatic device called Whistler LFMi is available, which in principle enables large-scale routine measurement of infant lung function.

In Asia, there is limited data of healthy infant lung function.[12] The aim of this study was to identify determinants of the distribution of healthy infant lung function in Indonesia and to compare these findings with those of a Western population.

Material and Methods

Study population

Lung function measurements were performed in healthy infants consecutively recruited in Budi Kemuliaan Hospital, a mother and child hospital in central Jakarta, prior to their immunization visit (figure 1). This study was approved by the medical ethics committee of the Faculty of Medicine of the University of Indonesia.

Infant lung function measurements of Indonesian infants were compared with Dutch infants of the WHISTLER study, which is an ongoing prospective birth cohort study in Utrecht, the Netherlands.[9] Within this study, lung function of healthy infants ≤2 months were measured using the same technique (SOT). Exclusion criteria of both studies were gestational age < 36 weeks, major congenital abnormalities and neonatal respiratory disease.

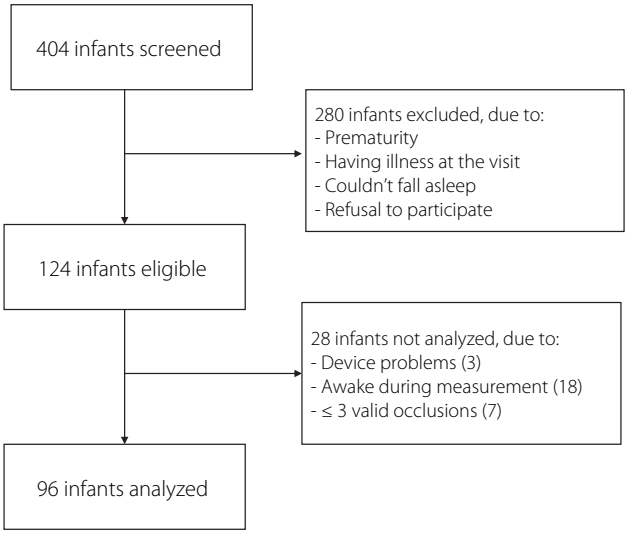

Figure 1. *Flow chart of subject recruitment*

Lung Function Measurement

Measurements were performed during natural sleep in a supine position with a neutral head and neck posture without the use of any sedation. Lung function was assessed from measurements of passive respiratory mechanics (Rrs, Crs and Trs of the total respiratory system) using the SOT. Briefly, end-inspiratory airway occlusion induces a Hering-Breuer reflex with complete relaxation of respiratory muscles and passive expiration after release of the occlusion. During periods of no flow, rapid pressure equilibration can be reached. The pressure at airway opening (Pao) represents the elastic recoil pressure of the respiratory system and can be related to changes in volume and flow in order to calculate the Crs and Rrs of the respiratory system. The slope of the descending portion of the passive flow-volume loop represents the Trs of the respiratory system.[7,8]

All measurements in Indonesia were performed using the automated Whistler LFMi (Medispirit BV, Nuenen, The Netherlands) meeting ERS/ATS criteria.[8,10,11] Airflow was measured by Whistler LFMi using ultrasonic airflow measurement (linear range 0-2 L/s, resolution 1 L/s, accuracy 2.5% or 2.5 mL/s, sample rate 160 Hz). Volume was obtained by integration of the airflow signal. Pressure changes at airway opening were measured with a pressure transducer built inside Whistler LFMi (range ±6.9 kPa, resolution 2 Pa, accuracy 1%, sample rate 1600 Hz). Dead space in this device is 11 ml. The data of Whistler LFMi was wirelessly interfaced with Bluetooth to a computer for real-time display, storage and analysis.

The WHISTLER study measured airflow by using a heated Lilly-type pneumotachometer (series 8300, linear range 0-10 L/min; Hans Rudolph Inc.,Kansas City, MO, USA). 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 was digitized with a sampling rate of 200 Hz and interfaced to a computer for real-time display, storage and analysis.[5,7]

The main difference between Whistler LFMi and the device used by the WHISTLER study is that the flow is automatically interrupted, while in the latter device the occlusion is performed manually. Occlusions were considered acceptable if they met the criteria of the ERS/ATS task force on infant lung function. Briefly, expiration needs to be smooth within 10% of the previous expiration and without evidence of glottis closure, braking or active expiratory effort, duration of a pressure plateau 100 ms and variability<10 Pa and linearity of the descending part of the passive flow-volume loop over at least 40% of expiration with r2>0.99.[8,11] At least three technically acceptable measurements were used to calculate the mean Crs, Rrs and Trs values. Both Indonesian and WHISTLER lung function data were calculated offline using a custom-built software package (Luna 1.6, Utrecht, the Netherlands)

Anthropometric Measurement

Body weight was measured using an infant scale (Tanita, Tokyo, Japan) and body length using an infant stadiometer.

Questionnaire

Guided interviewing with a questionnaire was used to record subject's characteristics, constitutional factors, and exposure to environmental factors. Subject characteristics measurements included age at visit, birth weight, birth length, gender, gestational age and thoracic circumference. Environmental factors consisted of tobacco smoke exposure during and after pregnancy and air pollution exposure which was represented by area of living. Area of living was classified into several regions of Jakarta (central, west, south or suburbs).

Statistical Analysis

The subject characteristics are presented in percentage, mean (SD) or median (25th quartile, 75th quartile). Univariable linear regression analysis was used to assess the associations of possible determinants with the lung function parameters Crs and Rrs as dependent variable. In a multivariable regression analysis we assessed the influence of maternal and environmental factors adjusted for weight of the child. We classified area of living into two distinct areas based on differences in air pollution. Suburbs and south Jakarta (reference) were compared to central and west Jakarta. Using a paired-t test, we compared the Crs and Rrs of nine infants measured with the Whistler LFMi and the Manual SOT device. Differences in Crs and Rrs of Western and Indonesian infants (<2 months) were tested using T-test for independent samples. Multivariable models with the product of weight and study population (WHISTLER versus the Indonesian study population) as interaction term were applied to study the trend of the linear regression coefficients. Statistical analysis was performed using SPSS Inc.,2011, Chicago USA, version 20.0 for Windows. A p-value <0.05 was considered statistically significant.

Results

An overview of the recruitment is showed in figure 1. Among the eligible subjects, valid infant lung function measurements were obtained in 77.4% of subjects. Healthy infants ranging in age between 1 to 10 months (median age 2.23 months) were measured using automated SOT to determine compliance (Crs) and resistance (Rrs) of the respiratory system. Table 1 describes the subject characteristics including anthropometry, constitutional and environmental factors. Our study showed a mean of 70.8 mL/kPa and 4.55 kPa/L/s for the Crs and Rrs respectively.

Table 2 shows the subject characteristics related to respiratory Crs and Rrs. Age, weight and length at visit were positively related to Crs and inversely with Rrs. With each kg increase in weight compliance increased with 12.70 mL/kPa (95% CI 9.44 to 15.95) and Rrs decreased with -0.47 kPa/L/s (95% CI -0.63 to -0.30). Sex was not associated with infant lung function.

Table 2. *General characteristics and lung function in Indonesian infants*

	Compliance (mL/kPa)			Resistance (kPa/L/s)		
	B	**95% CI**	**p-value**	**B**	**95% CI**	**p-value**
Age (days)	0.27	0.20, 0.34	<0.01	-0.01	-0.14, -0.07	<0.01
Weight at visit (kg)	12.70	9.44, 15.95	<0.01	-0.47	-0.63, -0.30	<0.01
Length at visit (cm)	2.88	2.12, 3.64	<0.01	-0.12	-0.15, -0.08	<0.01
Female sex	-3.00	-13.63, 7.64	0.58	-0.11	-0.60, 0.39	0.68

The numbers represent the linear regression coefficients with 95% confidence interval and p-values.

Table 3 shows the influence of environmental factors on lung function in infants. Tobacco smoke exposure during and after pregnancy was not associated with Crs or Rrs of the respiratory system. Infants living in central or west Jakarta had a lower Crs compared to children living in south Jakarta or suburbs (linear regression coefficient after adjustment for weight -12.61 mL/kPa, 95% CI -22.84 to -2.38). Rrs of the respiratory system was not associated with the area of living.

Table 3. *Environmental factors and lung function in Indonesian infants*

	Unadjusted			Adjusted for weight		
	B	**95% CI**	**p-value**	**B**	**95% CI**	**p-value**
Compliance (mL/kPa)						
Smoke exposure	-9.07	-22.62, 4.49	0.19	-0.66	-11.53, 10.21	0.90
Area of living[a]	-16.99	-29.87, -4.10	0.01	-12.61	-22.84, -2.38	0.02
Resistance (kPa/L/s)						
Smoke exposure	-0.07	-0.69, 0.56	0.83	-0.38	-0.94, 0.17	0.17
Area of living[a]	0.14	-0.47, 0.75	0.65	-0.03	-0.56, 0.51	0.92

[a]*Area of living: south Jakarta and suburbs were used as reference central and west Jakarta*

Measurements of lung function using both the manual SOT device and automated Whistler LFMi were done in 9 subjects at one occasion. There was a trend of a higher Crs and lower Rrs in the measurements with the Whistler LFMi compared to the manual SOT device, however this was not significant. The mean Crs using manual SOT device was 56.6 mL/kPa versus 61.5 mL/kPa with Whistler LFMi (p-value 0.32, paired t-test). The mean Rrs of the manual SOT device was 6.6 kPa/L/s versus 6.1 kPa/L/s with Whistler LFMi (p-value 0.39, paired t-test).

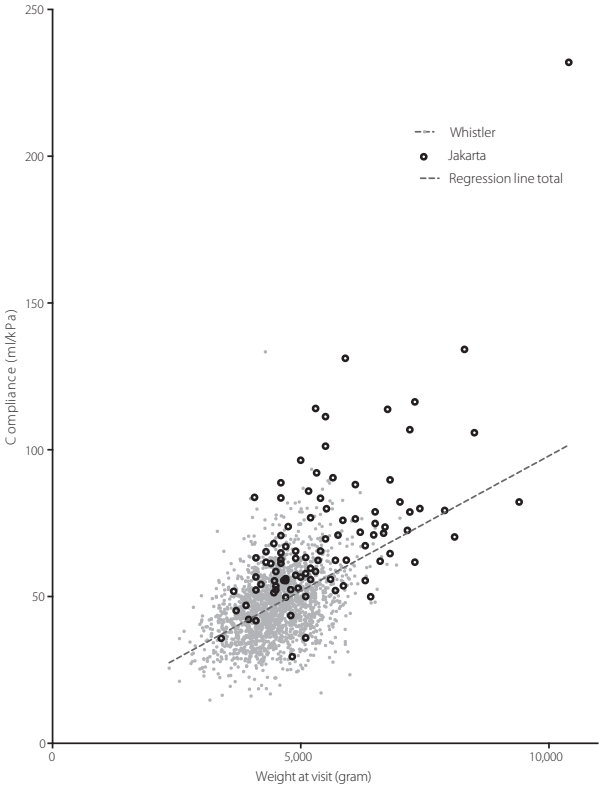

Figure 2. *Compliance of the respiratory system in Indonesian and Dutch infants by weight at visit*

Figure 2 and 3 shows scatter plots of the Crs and Rrs by weight for Indonesian and WHISTLER infants. As shown by the plot the Indonesian children had higher Crs and lower Rrs values compared to the WHISTLER children. The increase in Crs and decrease in Rrs with increasing weight was similar in Whistler and Indonesian infants ≤2 months (linear regression coefficients of the interaction term with Crs and Rrs were respectively -0,001, p-value 0,753 and <0,001, p-value 0,967).

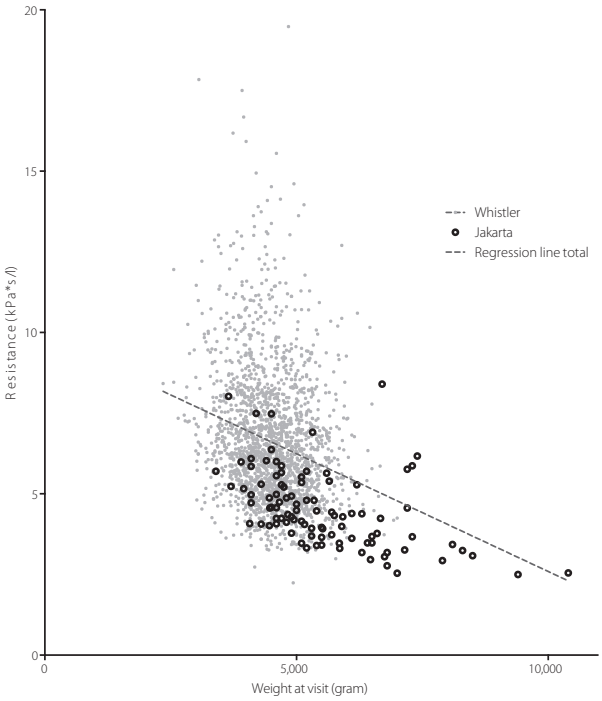

Figure 3. *Resistance of the respiratory system in Indonesian and Dutch infants by weight at visit*

Discussion

This is the first study, which describes determinants of lung function distribution in infants from Southeast Asia. Performance of these measurements showed a good feasibility and as described in western populations, age, weight and height are important determinants of infant lung function. Air pollution is an important public health problem in Jakarta and our study suggests that already in early infancy it has a negative influence on lung function, with clearly lower values of Crs in the polluted central and west Jakarta areas.

Previous studies measuring passive respiratory mechanics using manual SOT have been done and showed good feasibility (73% technically acceptable measurement) as well as good intra- and inter-observer variability.[7] Our study showed a similar feasibility with 77.4% technically acceptable measurements among eligible subjects. Some characteristics of our research need to be considered. We were not able to measure the Indonesian children with the same device as the WHISTLER children. We used the same technique, but a difference between the two devices was the automatic versus manual interruption of the flow. Although not significant, we saw within nine Dutch children measured with both devices a trend towards higher Crs and lower Rrs values of the Whistler LFMi device compared to the manual device used in the WHISTLER study. Therefore, we were not able to compare the values of

the lung function of these two populations. However, we do think it is reasonable to assume that the standard difference between the two devices did not affect the degree of increase in lung function with increasing age, which makes the comparison of this determinant on lung function distribution in these two populations possible. We used the address of the participant as an indicator for air pollution. A previous study among high school students of Jakarta showed that that the NO_2 and PM_{10} levels were higher in Central Jakarta versus South Jakarta. The NO_2 emission was associated with more respiratory complaints.[13] Central Jakarta is the center of business, which has more traffic than any other part of Jakarta and west Jakarta is a very crowded living area with many home industries. Furthermore, another study showed that the blood lead concentration in school children at central Jakarta was higher compared to south Jakarta.[14] These findings support our findings of a possible effect of air pollution on lung function in early life.

As in other studies, age, weight and height were predictors of lung function.[5] Higher age, weight and height were associated with higher Crs and lower Rrs. Our result enhance those findings since the age range in our study was broader compared to the WHISTLER study which only included infants until 8 weeks of age. While previous studies showed a reduced lung function in boys compared to girls,[5,15–18] our study is consistent with others who found no significant difference in lung function between boys and girls.[19]

Besides anthropometry, environmental factors seem to co-determine infant lung function as well.[18,20-22] In our study we could not demonstrate an association between tobacco exposure after birth with either lung compliance or resistance. Jakarta as a metropolitan city is well known for its pollution. Many studies have shown the effect of air pollution on lung function.[13,21–24] As far as we know, only one study is performed in infants.[22] This study, performed in Bern, Switzerland, used physiological surrogates for lung growth and development as outcome parameter. The results of this study suggest that prenatal exposure to air pollution might be associated with higher respiratory need and airway inflammation in newborns. In Jakarta, children are exposed to much higher levels of air pollution compared to the infants of the former study in Swiss. In our study we showed that already during infancy there is a large difference in lung function measured in children living in more polluted areas. The early postnatal period is important for lung development.[25] Early changes in lung function track into later life,[26] which could have a large impact on respiratory morbidity in adulthood. Especially in cities with much air pollution, like Jakarta, it's important to get a better understanding of the influence of air pollution on lung function in early life.

In conclusion, our results suggest that besides age, weight and height, air pollution is an important determinant of infant lung function in Southeast Asian children.

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Chapter 11

General discussion

Patient X was born on the 2nd of December 1931. During his first year of life, he repeatedly suffered from wheezing illnesses. At the age of 10 years, he was diagnosed with asthma. During a visit to the general practitioner at middle age his blood pressure was checked and appeared to be too high. He received antihypertensive medication. During the subsequent years he became increasingly dyspnoeic due to chronic obstructive pulmonary disease and at the age of 75 years he needed oxygen therapy. One year later he died from a myocardial infarction.

Cardiovascular and respiratory diseases often coexist within one individual. In this thesis we have explored the early origins of cardiovascular and respiratory development. The burden of cardiovascular and respiratory disease is high and the prevalence is expected to increase. To reduce this burden, implementation of appropriate prevention strategies is important. Knowledge about the shared development of non-communicable diseases and its risk factors is crucial to select the most effective prevention strategy. It is important to gain more knowledge about why these diseases occur together. In this thesis we present a model which presents potential associations that have to be studied to unravel the underlying mechanism of associations between the cardiovascular and respiratory system throughout life. These associations could lead to developmental changes during life, resulting in an increased risk of both cardiovascular and respiratory disease.

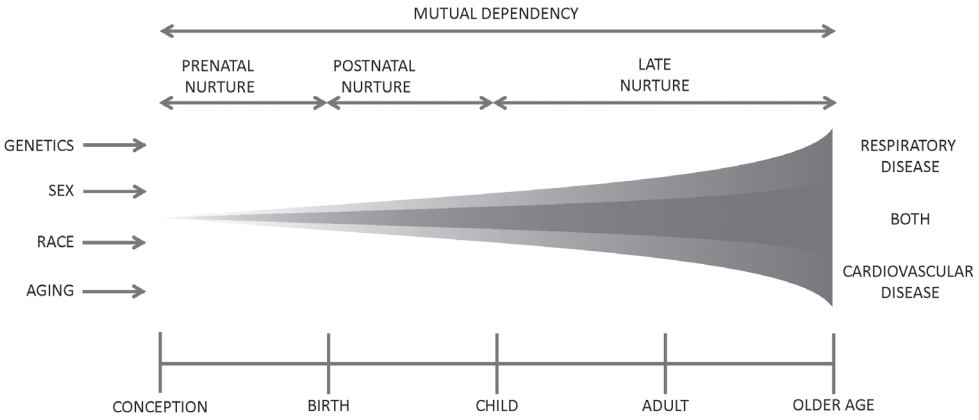

Figure 1. *A model which represents potential associations that have to be studied to unravel underlying mechanisms of associations between the cardiovascular and respiratory system.*

Nature

Genetics

Shared genetic linkage may explain the associations between cardiovascular and respiratory diseases. E.g. the CHRNA3 gene is associated with an increased risk of COPD and peripheral arterial diseases.[1,2] Whether this gene could explain part of the shared development of cardiovascular and respiratory

diseases is unknown. As far as we know, only one study examined the shared genetic relation between the respiratory and cardiovascular system. In this study among twins, no genetic covariance was found between FEV1, FVC and augmentation index.[3] The path to fully uncover the genetic basis of both respiratory and cardiovascular diseases has just begun. Future large-scale genome-wide association studies (GWAS) should explore linkage between these two diseases. Furthermore, the role of epigenetics is unexplored as well.

Sex and race

There are sex-specific and race-related differences in the manifestation of cardiovascular and respiratory diseases.[4, 5] Previous studies mainly focussed on men (or both sexes) and western populations. Whether the association between the cardiovascular and respiratory system differ according to race or sex is not fully known. It is important to gain more knowledge about women and populations from lower- and middle income countries, as in the past few years there is an increasing prevalence of non-communicable diseases and exposure to risk factors in these populations. Exposure to other risk factors in these populations could contribute to the development of diseases in these populations.

Aging

Mechanisms related to accelerated aging are involved in the pathogenesis of COPD and atherosclerosis. Telomere length is widely considered to be a marker of biological aging. If telomere length reaches a critical value, it will lead to cell cycle arrest and eventually apoptosis. Shorter telomeres, a marker of aging, is present in peripheral blood leukocytes of COPD patients compared with control patients.[6, 7] Shorter leukocyte telomere length is also associated with cardiovascular risk factors and common cardiovascular diseases, however causality remains undetermined.[8] Oxidative stress and inflammation could affect telomere length as well. Whether short telomeres play a role in the association between the cardiovascular and respiratory system is unknown.

Nurture

A wide range of environmental factors, which are often shared, affect chronic diseases. Several exposures in early life have also shown to affect both the developments of the cardiovascular and the respiratory system. The prenatal and early postnatal period is a vulnerable period as specific tissue formation and cell differentiation takes place. Disease risk in adulthood can be induced already in this developmental period and can be modified by nutrients and environmental exposures. Different environmental factors could play a role in prenatal, postnatal and adult life.

Prenatal nurture

Early evidence from the developmental origins hypothesis is derived from studies exploring the role of fetal under nutrition. A higher risk of heart disease was observed in people with lower birth weights.[9, 10] The negative consequences of a suboptimal intrauterine nutritional environment on cardiovascular

health are also observed in studies that showed associations with higher maternal weight or body mass index (BMI). A higher maternal weight is associated with increased risk of cardiovascular events in adulthood, but also with precursors of cardiovascular disease earlier in life, like a higher systolic and diastolic blood pressure.[11–13] Animal studies have shown that a higher maternal weight could also affect cardiac function.[14] In chapter 5 we confirmed this finding in childhood. We found evidence for an impaired systolic cardiac function among offspring of mothers with a higher maternal BMI. More recently, research about intrauterine nutritional environment has focused on the consequences on respiratory health as well. Higher maternal weight has been associated with increased risk of wheezing illnesses in early life.[15–17] In chapter 6 we confirmed these findings and explored if neonatal lung function could explain the associations. The associations of maternal BMI and wheezing in the first year of life could partially be explained by neonatal lung function. Up to the age of 5 years, associations between maternal BMI and consultations and prescriptions for respiratory illnesses were present, but these could not be explained by a reduced lung function in infancy. The mechanisms underlying these associations remain unclear. It would be interesting to study if hormones with a higher circulating level in obesity, like leptin, could play a role in these associations.

Postnatal nurture

The most well known modifiable risk factor in adulthood, smoke exposure, negatively affects both systems in early life as well. Maternal smoking during pregnancy is associated with thicker intima media thickness of the carotid artery, lower distensibility and a higher systolic blood pressure in the offspring during childhood.[18–20] For the respiratory system, associations has been described with an impaired lung function and an increased risk of wheezing.[21, 22]

Another determinant of development of both the respiratory and cardiovascular system is postnatal weight gain. Rapid postnatal weight gain is associated with more wheezing illnesses in the first year of life and a decreased lung function and thicker arterial walls at the age of five years.[23–25] Other evidence suggests a role of exposure to infections. Acute infection in childhood is associated with impaired endothelium-dependent vasodilatation, which is recognized as a key initiating event in the development of atherosclerosis.[26] Lower respiratory tract infections experienced in early childhood are associated with reduced lung function measurements in adulthood.[27] However, there is still debate whether infections lead to lung damage or whether children who experience infections have a pre-existing lung function reduction.

Late nurture

Prolonged continuous exposure to risk factors form a major contribution to the development of both cardiovascular and respiratory diseases. Although previous studies did adjust for the most important risk factors, like smoking, socio-economic status, age and gender, there could still be some unmeasured residual confounding. Preferably, a population-based prospective cohort study has to be performed, in which children are followed from prenatal to adult life. However, the large sample size and the long

period needed for follow-up is a major limitation of this design. Studies based on preclinical alterations, i.e. surrogate end points, may circumvent these limitations provided that their relationship with end points, like cardiovascular and respiratory morbidity and mortality are scientifically proven. As the oldest children of our birth cohort are currently 13 years, it will take a long time before hard end points will occur. Till then, the surrogate markers will provide us the best available evidence.

Mutual dependency

We have shown that associations between the cardiovascular and respiratory systems, as described in adulthood, are not detectable in healthy children (chapter 2 and 3). In healthy children a more favourable lung function was associated with a higher blood pressure and increased arterial stiffness, but that was largely explained by anthropometry. Associations between an adverse cardiovascular profile and an impaired lung function develop later in life. We, and others, mainly focused on spirometric indices as a parameter of the pulmonary system without information on lung volume or diffusing capacity. These measures could provide useful information as well.

In contrast to evidence from healthy children, in children with a disease, specifically when characterized by severe pulmonary problems, such as cystic fibrosis, we found evidence for subclinical cardiac dysfunction and increased arterial stiffness (chapter 4). It would be interesting to study if cardiovascular changes are present in children with other chronic respiratory diseases as well.

In children with a congenital heart disease abnormal lung development has been described as well. Recent studies suggest that this could be a consequence of altered pulmonary flow, which could lead to pulmonary remodelling. It is unknown if this hypothesis derived from models with a disease could be extrapolated to healthy individuals as well. As far as we know, no studies have been performed in healthy children to study the association between cardiac and pulmonary function.

Mechanisms

Whether the changes in cardiovascular and respiratory development are directly causally related is not fully clear. Many cross-sectional studies have been performed, which limits causal inference. Two previous longitudinal studies among adults have shown that a decline in lung function predicted the development of atherosclerosis and hypertension.[28, 29] The direction of this association was specific for lung function change predicting the development of hypertension. Changes in blood pressure did not predict loss of lung function.[28] A study of the effects of respiratory interventions on the cardiovascular system could give further insights into the mechanisms underlying these associations.

Several pathophysiological processes could lead the development of both diseases within one individual. Previous studies in cystic fibrosis, chronic obstructive pulmonary disease and asthma, all showed evidence for an association between inflammation and the degree of cardiovascular changes.[30–32] Patients with chronic respiratory diseases experience recurrent pulmonary infections. In patients with cystic fibrosis these recurrent infections are already present in early life, which might explain why

cardiovascular changes in these patients are present in an early stage of the disease. Recurrent infections are accompanied by higher circulating levels of pro-inflammatory mediators, which could potentially decrease elasticity of both vasculature and lung tissue. Low-grade systemic inflammation is thought to be important in the initiation and progression of the development of atherosclerotic lesions, the underlying cause of cardiovascular disease.[33] Pro-inflammatory mediators could also affect cardiac contractility and arterial stiffness.[34, 35]

Other factors than inflammation in patients with a chronic respiratory disease could affect the cardiovascular system as well. Chronic hypoxia and oxidative stress, which is present in patients with COPD, could contribute to the development of atherosclerosis.[36] Hyperinflation of the lungs and elevated pulmonary arterial pressures could affect cardiac function, ventricular filling and venous return. Medication used for COPD or asthma, like corticosteroids or beta-agonists, and antihypertensive medication could also play a role in the development of diseases.[37–40] Future research should be performed to further elucidate the underlying mechanism.

Concluding remarks

The cardiovascular and respiratory system are closely intertwined. In this thesis we have shown that associations between the cardiovascular and respiratory system become apparent at an older age or earlier if a disease of one of these systems is present. In addition, they share many risk factors throughout life. These suggest that it is important for future research to study the cardiovascular and respiratory system in parallel. Especially in our current health care system, in which physicians are increasingly trained to become a specialist in a particular area, it is important to gain more awareness among physicians of the shared origins and parallel development of the cardiovascular and respiratory system. Knowledge of the early origins of both cardiovascular and respiratory diseases might provide new opportunities for prevention initiatives. Lowering or prevention of exposure to risk factors may prevent or postpone processes. Many lifestyle risk factors originate in youth, so it is important that prevention strategies focus on the earliest phase of life and even before pregnancy takes place. Therefore, paediatricians, maternal and child health professionals could play an important role in preventing the development of diseases, before their clinical occurrence.

It is clear that adverse exposures in early life could lead to more than one disease over the lifespan. A focus on one disease may significantly underestimate the consequences of exposure to one risk factor. Therefore, prevention strategies should be established with a focus on more than one disease.

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Chapter 12

To conclude

Summary

Non-communicable diseases are the major causes of death and disability worldwide and the prevalence of these diseases are expected to increase substantially in the future. Chronic diseases are often studied separately, but there is growing awareness that these diseases are closely linked. In this thesis we focused on two non-communicable diseases, cardiovascular and respiratory diseases, which form a large contribution of the total morbidity and mortality of chronic diseases. Epidemiological studies have shown that these diseases often co-exist within the same patient, independent of shared risk factors like smoking. To gain more insights into shared risks factors and the origin of the interaction between the respiratory and cardiovascular system, we performed our research in childhood, as increasing evidence suggests that these chronic diseases have their origins in early life. Adverse exposures in utero and childhood could lead to structural or functional changes in organ systems and due to tracking of these changes, it could make them more susceptible for cardiovascular or respiratory diseases in later life.

Interaction of the cardiovascular and respiratory system

In the first part of this thesis we explored whether interactions of the cardiovascular and respiratory system are already present in early life. We have shown that associations between the cardiovascular and respiratory system, as described in adulthood, are not present in healthy children (**chapter 2 and 3**). In healthy children a more favourable lung function was associated with a higher blood pressure and increased arterial stiffness, which is largely explained by anthropometry. Associations between an adverse cardiovascular profile and an impaired lung function develop later in life. This is confirmed in **chapter 2** in which we have shown that the association reversed with increasing age. In contrast to evidence from healthy children, in children with a disease, which is characterized by severe pulmonary problems, cystic fibrosis, we found evidence for subclinical cardiac dysfunction and increased arterial stiffness (**chapter 4**).

Shared determinants

In the second part of this thesis we focussed on determinants, which could affect both the cardiovascular and respiratory development in early life. The prenatal and early postnatal period is a vulnerable period as specific tissue formation and cell differentiation takes place. Disease risk in adulthood can be induced already in this developmental period and can be modified by nutrients and environmental exposures. Weight is an important modifiable risk factor for both respiratory and cardiovascular disease. In **chapter 5 and 6** we studied the association between maternal relative weight and the cardiovascular and respiratory system, respectively.

In **chapter 5** we observed a trend of reduced systolic cardiac function with increasing maternal BMI. Although subtle, these findings adds to and extends accumulating evidence which proposes that adverse conditions in utero might lead to lifelong changes in body composition and physiology, which could lead to adverse health in adulthood.

Higher maternal weight has also been associated with increased risk of wheezing illnesses in early

life. In **chapter 6** we confirmed these findings and explored if neonatal lung function could explain the associations. The associations of maternal BMI and wheezing in the first year of life could partially be explained by neonatal lung function. Till the age of 5 years, associations with consultations and prescriptions for respiratory illnesses are present, but these could not be explained by a reduced lung function in infancy. The mechanisms underlying these associations remain unclear.
Several studies have proposed that leptin could play a role in these associations. Leptin is a hormone that is primarily produced by adipocytes and it correlates strongly with percentage of body fat. In adults, higher leptin levels are associated with increased risk of coronary heart disease, especially in males. Leptin and leptin receptor system expression and signalling is altered in the airways of patients with asthma and COPD. Higher leptin levels have also been associated with an impaired lung function in adulthood. In **chapter 7** we explored if leptin levels in childhood are also associated with lung function. In our cross-sectional study among 8-year-old children, we observed an association between higher leptin levels and impaired lung function.

Evaluation of new non-invasive devices

Hard end points of chronic cardiovascular and respiratory disease only will become apparent in adulthood. Non-invasive tests to assess the cardiovascular or respiratory system could facilitate the detection of children at risk for cardiovascular or respiratory diseases. Assessment of these systems in early childhood is challenging. In the last part of this thesis, we studied new non-invasive measurement devices, which have the potential to evaluate cardiovascular and respiratory risk in youth.
In **chapter 8** we evaluated feasibility of measurements of arterial stiffness (pulse wave velocity and augmentation index of the aorta) with a new non-invasive device in preschool children. Feasibility of measurements in this age group is moderate. Height was the most important determinant of augmentation index in these children.
In children from 5 years of age, spirometry is a reliable and valid method to assess the respiratory system. However, as children need to be able to control their in- and exhalation, it is not suitable for preschool children. In **chapter 9 and 10** we evaluated two non-invasive devices, which can be used in infants and preschool children. In **chapter 9** we assessed a nocturnal wheeze measurement device, which could provide an objective measure of symptoms in these children. Measurements were performed in healthy preschool children. In almost 50% of these children sporadic wheeze was observed during the night. Although higher nocturnal wheeze rates were related to increased respiratory system resistance, it was not related to clinical wheezing symptoms. In **chapter 10** we assessed a device, which is able to measure compliance and resistance of the respiratory system in infancy using the single occlusion technique. We performed these measurements in Indonesian infants. Exposure to other risk factors than the western population could contribute to the development of diseases in these children. Our results suggested that besides age, weight and height, air pollution is an important determinant of infant lung function in Southeast Asian children.

Chapter 11 provided a general discussion with implications for future research and clinical practice.

Samenvatting

Chronische ziekten komen erg veel voor en zijn een belangrijke oorzaak van ziekte en sterfte op de volwassen leeftijd. Het aantal mensen wat lijdt aan een chronische ziekte neemt naar verwachting de komende jaren toe. Chronische ziekten worden vaak separaat bestudeerd, maar er is toenemende bewustwording dat verschillende chronische ziekten nauw met elkaar verbonden zijn. In dit proefschrift focussen we op hart- en vaatziekten en chronische ziekten van de longen. Epidemiologische studies hebben aangetoond dat deze ziekten vaak samen voorkomen bij dezelfde patiënt, onafhankelijk van gemeenschappelijke risicofactoren als roken. Om meer inzicht te krijgen in de oorsprong en samenhang tussen deze ziekten hebben we onderzoek gedaan bij kinderen, omdat er toenemend bewijs is dat deze chronische ziekten hun oorsprong al op jonge kinderleeftijd hebben. Blootstelling aan risicofactoren in de baarmoeder of tijdens de eerste levensjaren kunnen leiden tot structurele of functionele veranderingen in organen, waardoor op latere leeftijd het risico op chronische ziekte is verhoogd.

Samenhang tussen hart- en bloedvaten en de luchtwegen

In het eerste deel van dit proefschrift hebben we onderzocht of de samenhang tussen hart- en bloedvaten en de luchtwegen die op oudere leeftijd wordt beschreven, ook aanwezig is bij jonge kinderen. Volwassenen met een slechtere longfunctie hebben vaak stijvere vaten en een hogere bloeddruk wat een hoger risico geeft op hart- en vaatziekten. Uit dit proefschrift blijkt dat dit bij gezonde kinderen niet zo is (**hoofdstuk 2 en 3**). Bij gezonde kinderen is een betere longfunctie geassocieerd met een hogere bloeddruk en stijvere vaten, wat grotendeels verklaart kan worden door lengte en gewicht van het kind. Bij kinderen met taaislijmziekte (cystic fibrosis), een ziekte die gepaard gaat met veel longproblemen, waren wel aanwijzingen voor een verminderde knijpfunctie van het hart en een verhoogde vaatstijfheid in vergelijking met gezonde kinderen (**hoofdstuk 4**).

Gemeenschappelijke factoren

In het tweede deel van dit proefschrift hebben we factoren bekeken die zowel de ontwikkeling van hart- en bloedvaten als de ontwikkeling van de luchtwegen kunnen beïnvloeden. Gewicht is op de volwassen leeftijd een risicofactor voor zowel het ontstaan van hart- en vaatziekten als chronische ziekten van de longen. In **hoofdstuk 5 en 6** hebben we de relatie bestudeerd tussen het gewicht van de moeder en de ontwikkeling van de luchtwegen en het hart- en de bloedvaten van jonge kinderen. In **hoofdstuk 5** observeerden we een trend van verminderde knijpkracht van het hart bij het kind naarmate de BMI (body mass index, een maat voor overgewicht) van moeder hoger was. Deze bevinding was subtiel, maar draagt bij aan het toenemende bewijs dat ongunstige omstandigheden in de baarmoeder kunnen leiden tot structurele veranderingen in organen.

Hoger gewicht van de moeder wordt ook in verband gebracht met een verhoogd risico op piepklachten in de eerste levensjaren van het kind. In **hoofdstuk 6** bevestigen we deze bevindingen en hebben we daarnaast gekeken of dit verklaard zou kunnen worden doordat deze kinderen mogelijk een lagere longfunctie hebben. De relatie tussen de BMI van moeder en piepklachten in het eerste levensjaar

kon gedeeltelijk worden verklaard door de longfunctie na de geboorte. Op 5-jarige leeftijd zagen we dat kinderen van moeders met een hoger BMI vaker de huisarts bezochten voor piepklachten en vaker medicijnen voor deze klachten kregen voorgeschreven. Dit kon niet worden verklaard door een lagere longfunctie. Het mechanisme wat aan deze relaties ten grondslag ligt is nog onduidelijk.
Verschillende studies hebben geopperd dat leptine een rol zou kunnen spelen in deze relaties. Leptine is een hormoon dat voornamelijk wordt geproduceerd door vetcellen. Volwassenen met een hoger leptine gehalte in het bloed hebben een hoger risico op hart- en vaatziekten, vooral bij mannen. Leptine blijkt ook aanwezig te zijn in de longen. Een hoger leptine gehalte is bij volwassenen geassocieerd met een lagere longfunctie. In **hoofdstuk 7** hebben we gekeken naar de relatie tussen het leptine gehalte in het bloed bij 8-jarige kinderen en de longfunctie. We zagen dat kinderen met een hoger leptine gehalte een lagere longfunctie hadden.

Evaluatie van nieuwe meetinstrumenten
Een hartaanval of een beroerte treedt meestal pas op latere leeftijd op. Nieuwe meetinstrumenten waarmee op een niet-belastende manier het hart- en de bloedvaten of de luchtwegen kunnen worden gemeten, kunnen het mogelijk maken om kinderen met een verhoogd risico voor het ontstaan van chronische ziekten te detecteren. Het uitvoeren van metingen op de jonge kinderleeftijd is uitdagend. In het laatste deel van dit proefschrift bestuderen we nieuwe apparaten die in potentie het hart en de bloedvaten of de luchtwegen op jonge leeftijd goed kunnen evalueren.
In **hoofdstuk 8** evalueerden we de haalbaarheid van vaatstijfheidsmetingen bij 3-jarige kinderen met een nieuwe niet-belastende methode. De uitvoerbaarheid van metingen op deze leeftijd is matig. Daarnaast hebben we onderzocht welke factoren gerelateerd zijn met vaatstijfheid. Lengte bleek een factor te zijn die geassocieerd is met vaatstijfheid bij deze jonge kinderen.
Vanaf 5-jarige leeftijd is het mogelijk om een longfunctiemeting (spirometrie) te doen waarmee een betrouwbare maat wordt gegeven van de longfunctie. Omdat bij deze meting kinderen in staat moeten zijn om de in- en uitademing te controleren is het niet toepasbaar voor jongere kinderen. In **hoofdstuk 9 en 10** evalueerden we twee nieuwe meetinstrumenten die wel kunnen worden gebruikt in deze jongere groep kinderen. In **hoofdstuk 9** evalueerden we een meetinstrument waarmee nachtelijke piepgeluiden van de longen worden gemeten. Dit zou een objectieve maat kunnen zijn voor symptomen bij jonge kinderen. Metingen werden uitgevoerd bij gezonde 3-jarige kinderen. In bijna 50% van deze kinderen bleek er sporadisch sprake te zijn van piepen gedurende de nacht. Ondanks dat het nachtelijk piepen bij deze kinderen gerelateerd was aan de weerstand van de luchtwegen, bleek het niet overeen te komen met de klinische symptomen. In **hoofdstuk 10** hebben we een meetinstrument onderzocht waarmee de longfunctie op de babyleeftijd kan worden gemeten. Deze metingen hebben we uitgevoerd bij Indonesische kinderen. Blootstelling aan andere risicofactoren dan de westerse wereld zou een rol kunnen spelen in het ontstaan van ziekte bij deze kinderen. Onze resultaten suggereerden dat naast leeftijd, gewicht en lengte, ook luchtvervuiling een belangrijke factor zou kunnen zijn die de longfunctie bij Indonesische kinderen zou kunnen beïnvloeden.

Hoofdstuk 11 bevat een algemene discussie met ideeën voor vervolgonderzoek en de klinische praktijk.

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List of publications

Eising JB, van der Ent CK, van der Gugten AC, Evelein AMV, Numans NE, Grobbee DE, Uiterwaal CSPM. Life-course of cardio-respiratory associations. Eur J Prev Cardiol. 2013 Oct 30.

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Eising JB, van der Ent CK, Teske AJ, Vanderschuren MM, Uiterwaal CSPM, Meijboom FJ. Comprehensive echocardiographic analysis in patients with cystic fibrosis reveals reduction in left and right ventricular function already at an early age. Submitted

Eising JB, Meijboom FJ, de Wilde MA, van der Ent CK, Uiterwaal CSPM. Maternal BMI and cardiovascular development in childhood. Submitted

Eising JB, Uiterwaal CSPM, van der Ent CK. Maternal body mass index, neonatal lung function and respiratory symptoms in childhood. Submitted

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Vanderschuren MM, Uiterwaal CSPM, van der Ent CK, **Eising JB.** Feasibility and characteristics of arterial stiffness measurement in preschool children. Submitted

Eising JB, Indawati W, Uiterwaal CSPM, Grobbee DE, Sapriani I, van der Ent CK. Characteristics of Southeast Asian infant lung function. Submitted

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Veldhoen ES, Schouten ES, **Eising JB**, Schouten AN, van Vught AJ, Bollen CW. Predicting adverse events in paediatric critical care: a prospective study. Submitted

Curriculum Vitae

Jacobien Eising was born on February 27th 1985 in Tuk, a small village near Steenwijk in the Netherlands. She graduated from secondary school in 2003 at the Regionale Scholen Gemeenschap Tromp Meesters in Steenwijk. At that same year she started her medical school at the Utrecht University. During her medical school she participated in research at the paediatric intensive care. She graduated in March 2010. After graduation she worked for a short period as resident at the department of Paediatrics at the Waterlandziekenhuis in Purmerend, the Netherlands. In June 2010 she started working on the research described in this thesis under supervision of Prof. Dr. C.K. van der Ent and Dr. C.S.P.M. Uiterwaal. During this work she collaborated with paediatricians of the University of Indonesia, visited Jakarta in order to obtain neonatal lung function data and obtained her Master of Science degree in Clinical Epidemiology in 2013 at the Utrecht University. In April 2014 she started working as a resident in Pediatrics in TerGooi Ziekenhuis in Blaricum under supervision of Dr. B.E. van Ewijk.

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