NIKOLAJEV KARI

The Effects of Intrauterine Growth Retardation on Lung Function at School Age

Doctoral dissertation

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ABSTRACT

Background: There is a lack of knowledge about the long-term respiratory outcome of low birth weight infants in general, this being especially the case for in utero growth retarded children. The present study focuses on the effects of intrauterine growth retardation on respiratory morbidity and lung function at school age. The study design is a prospective follow-up of twin pairs with discordant intrauterine growth patterns from school age throughout puberty.

Patients and methods: All children from multiple pregnancies were selected from 1978 to 1985 in a geographically defined area (North-Savo hospital district). Of them, 53 children constituting 19 intrauterinally disproportionally grown twin pairs were followed-up from birth up to the median age of 16 years (range 13-21). Lung function measurements and bronchial challenge test were performed twice in 53 children, at a median of 10 years of age and six years later. Pulmonary morbidity, lung function and challenge test results were studied in relation to intrauterine growth and other peri- and postnatal factors.

Results: The lung volumes were not associated with gestational age or with gestational age adjusted birth weight. With respect to the expiratory flows, FEF50 values were lower in the intrauterine growth retarded (IUGR) than in the appropriately grown (AGA) children.

Sixteen (25 %) of the 63 children had bronchial hyperresponsiveness (BHR) to cold air. Ten (15 %) of the 67 children had BHR in a methacholine challenge (MIC) test. Invasive ENT (Ear-nose-throat) procedures had been done significantly more often in the cold air responders than in the nonresponders. Doctor-diagnosed respiratory infections, numbers of antibiotic courses, episodes of otitis media and the need for ENT surgery were more common in the MIC responders than in the non-responders.

Three challenge tests, i.e. cold air inhalation, methacholine inhalation and an outdoor exercise-bronchodilatation test, were performed in 29 children. Eighteen (62 %) subjects exhibited positive responses in one or more of the tests, these being subdivided as 11/14 in the IUGR and 7/15 in the AGA groups (ns). Ten (34 %) children responded in two tests, and seven (70%) of these children belonged to the IUGR group. In addition, the two children responding in all three test were IUGR cases. The cold air responses were associated with responses in the outdoor exercise-bronchodilatation test, but not with methacholine reactivity.

BHR to cold air remained rather similar in the smaller and larger twin pairs after a follow-up period. However, all of the four cold responders at adolescence belonged to the smaller twin pairs. The reactivity to cold diminished during adolescence, though only in AGA children. The cold air challenge test and free running test outdoors identified generally the same responding children. The free running test outdoor needs no special facilities and is easy to do in everyday clinical practice, and it is recommended as a primary approach for children in whom a bronchial challenge test is indicated.

Conclusion: After rather mild neonatal pulmonary morbidity in an only modestly preterm twin population, as was the case in the present study cohort, IUGR does not seem to cause any major harm to lung health or function at school age or during adolescence.

Similarly, no differences were found in the reactivities to methacholine, cold air and free running between IUGR and AGA children. However, 60 % of the MIC-responders and 70 % of the responders to two or three challenges were earlier IUGR children. Thus, preliminary evidence was found that IUGR may be associated with BHR at school age.

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Medical Subject Headings: fetal growth retardation; infant, small for gestational age; infant, premature; child; twins; lung growth and development; birth weight; lung volume measurements; bronchial hyperreactivity; spirometry; exercise tests; cold; methacholine tests; follow-up studies; prospective studies
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Kuopio, May, 2003

Kari Nikolajev
ABBREVIATIONS

AGA = Appropriate growth for gestational age
BHR = Bronchial hyperresponsiveness
BPD = Bronchopulmonary dysplasia
CLD = Chronic lung disease
EIB = Exercise induced bronchospasm
FEV1 = Forced expiratory volume in one second
FEF25-75 = Forced mid-expiratory flow
FEF50 = Forced expiratory flow at 50 % of FVC
FEV% = FEV1/Forced vital capacity ratio
FRT = Free running test
FVS = Flow volume spirometry
IHCA = Isocapnic hyperventilation of cold air
IUGR = Intrauterine growth retardation
LBW = Low birth weight (< 2500 g)
MIC = Methacholine inhalation challenge
PDA = Patent ductus arteriosus
PEF = Peak expiratory flow value in spirometry
PFT = Pulmonary function test
RDS = Respiratory distress syndrome
VLBW = Very low birth weight (< 1500 g)
WPEF = Peak expiratory flow value (Wright’s peak flow meter)
LIST OF ORIGINAL PUBLICATIONS

This work is based on the following articles, which are referred to in the text by their Roman numerals.


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1. INTRODUCTION

Recent advances in neonatal intensive care have substantially improved the survival prospects of low birth weight infants (1). Respiratory morbidity following neonatal respiratory distress, especially in those born prematurely, is common during the first years of life (2), though it can also occur later (3). There is a lack of knowledge about the long-term respiratory outcome of low birth weight infants in general, in particular, very little is known about in utero growth retarded children.

"Prenatal programming" due to intrauterine growth retardation (IUGR) has been claimed to predispose surviving infants to abnormal lung function and chronic obstructive lung disease in adults (4). Barker published the hypothesis of the prenatal programming of pulmonary health in years 1986 to 1989 (5-8), and this hypothesis has been thereafter the subjects of intense debate (9, 10).

The mechanism of the prenatal programming of pulmonary health has remained thus far unresolved. One theory claims that IUGR may lead to diminished lung and airway growth, resulting in small calibers of the airways, and in the small volume of lung tissue. Alternatively, IUGR may increase bronchial reactivity due to the disproportional growth of airways and lung tissue. Thus far, Barker's hypothesis is only a theory, and more information is needed to either prove or disprove it.

There are retrospective epidemiological data suggesting that the birth weight may be a more important factor than the duration of gestation with respect to the incidence of later respiratory disease (11, 12). Among low birth weight (LBW) infants, respiratory distress syndrome (RDS)
and mechanical ventilation both increase respiratory morbidity after the neonatal period (13). In addition, even without IUGR prematurity intrinsically may, lead to small-sized airways, and further to bronchial obstruction or increased reactivity (14, 15). There is a lack of prospective follow-up studies in appropriate birth cohorts, which would have taken both birth weight and the duration of gestation into account.

In earlier studies, birth weight alone has been used as a measure of intrauterine growth (16). However, birth weight is strongly dependent on gestational age. Since prematurity itself influences later pulmonary development, function and morbidity, the effects of intrauterine growth and prematurity must both be taken into account, but it would be desirable to assess their effects separately.

The present study focuses on the effects of IUGR on respiratory morbidity and lung function at school age, including lung volumes, expiratory flows and bronchial reactivity. Our study design is a follow-up of twin pairs with discordant intrauterine growth patterns from birth to school age, these where then further followed throughout puberty.
2. REVIEW OF THE LITERATURE

2.1. Collection of the literature

The review of the literature was based on a computer assisted search of publications until the end of 2002. The abstracts and reviews were searched from two databases: the Cochrane database of systematic reviews (http://www.update-software.com/clibhome/clib.htm), and the MEDLINE database supported by National Library of Medicine (NLM) of the United States. MEDLINE citations and abstracts are available as the primary components of NLM’s PubMed database, which is also searchable via the Internet (http://www.ncbi.nlm.nih.gov/entrez). MEDLINE includes references of articles indexed from 1966 to the present. The coverage of Cochrane and Medline databases are worldwide, but only English sources were included in this review.

The following keywords were used in the search from the databases: IUGR, small for gestational age (SGA), lung function, outcome, child, adolescent, twins, prematurity, bronchial hyperreactivity, airway reactivity, bronchial hyperresponsiveness (BHR), bronchial provocation, methacholine.
2.2. Lung growth, development and remodeling

Growth of the lung tissue does not occur at a constant rate during fetal maturation. Airway development begins approximately 24 days after fertilisation, and the airways are complete by 16-20 weeks of gestational age (17). Alveolar development, which begins at around 20 weeks of gestational age, continues until the age of 2-3 years (18). In addition, the alveoli continue to increase in volume until lung growth ceases in early adult life (19). Growth retardation during mid-gestation may lead to a deranged pattern of lung growth, resulting in airways which are narrow in relation to the parenchymal size (20).

Given this pattern of lung development, prenatal and early postnatal factors have important effects on later airway function throughout childhood into adult life. Furthermore, any factor in childhood that impairs significantly normal airway growth is likely to affect adult lung function in an adverse manner (19).

The processes involved in the repair of damaged airways are called collectively “remodeling”. Remodeling can affect a number of important structural and functional elements of the airways including epithelium, smooth muscle, extracellular matrix, and mucus secretion (21). Many studies have suggested that remodeling is an important process in the development and persistence of increased airway reactivity. However, some infants appear to be predisposed to BHR and subnormal pulmonary function even prior to being confronted by an insult (22, 23), and accordingly, subnormal lung function if present at birth, may predispose to wheezing respiratory illnesses in the first years of life (24).
2.3 Programming of the fetal events

In the intrauterine stage, the organs of the body go through “critical” periods of development (25), and these periods reflect the times of rapid cell division. “Programming” describes the process whereby a stimulus or insult during this critical period has long-lasting or even lifelong effects (25). Undernutrition is a typical factor which programmes the human body and has long-term consequences. Some of the body’s “memories” about early undernutrition may become translated into pathological changes, and finally may form the basis for the appearance of diseases in later life (26).

The human fetus adapts to undernutrition by metabolic changes, by redistributing blood flow and by changes in the production of fetal and placental hormones which control growth (27). The immediate metabolic response of the fetus to undernutrition is catabolism: the fetus consumes her/his own tissues to provide energy (28). A prolonged undernutrition leads to slowing of growth. A growth retardation during the third trimester of gestation leads to disproportions in the sizes of the organ. Those organs and tissues, such as the lungs, that are growing rapidly at that time, are affected to the greatest extent.

By slowing the growth rate, the fetus protects organs that are critical for immediate survival, the brain in particular. The brain can be protected by redistributing the blood flow (29). For example, fetal hypoxemia in a sheep is associated with a 40% increase in cerebral oxygen delivery, whereas the pulmonary oxygen delivery is reduced by about 70% (30). This adaptation is known to occur in many mammals and probably also takes place in humans. Thus, the human fetus responds to undernutrition by sustaining the growth of the brain at the expense of the growth of the visceral organs, probably including also the lungs (31).
Nutrition has profound effects on the levels of fetal hormones (28). Although undernutrition leads to a fall in the concentrations of insulin and other hormones that control fetal growth, it also leads to an increase of cortisol production (32), a hormone which has marked effects to impair cell differentiation (27, 33).

The data from infants in Tucson (34) and Perth (22, 23) demonstrate that in utero or genetic factors contribute significantly to lung function and pulmonary morbidity in later childhood. These factors, for example, maternal smoking, affect airway development resulting in airways that are relatively small in diameter and have increased compliance, and thus are susceptible to obstruction (35).

2.4. Barker's hypothesis

The possible fetal origin of diseases has been recognised for more than 15 years in the relationship between birth weight and cardiovascular morbidity in adults in the UK (5, 8), and this recognition led later to the so-called Barker’s hypothesis. In these initial reports, the regional distribution of ischaemic heart disease in England and Wales reflected regional variations during nutrition in early life (5). Thus, Barker’s hypothesis initially was restricted to the prenatal “programming” of cardiovascular diseases.

2.4.1. Data supporting the hypothesis

The Barker’s “programming” theory was originally based on a population living in Hertfordshire, England, followed-up from the year 1911 onwards. Health visitors recorded the birth weights of all babies born in Hertfordshire, visited the homes periodically throughout the
infancy, and the records of these visits were preserved. Follow-up studies in men and women born 60 or more years earlier showed that lower weights at birth and at one year of age were associated with higher death rates from ischaemic heart disease, stroke and chronic obstructive pulmonary disease (4).

Barkerˇs initial reports from the years 1986-1991 have led to a growing interest in how prenatal events can influence the expression of diseases later in adult life. For lung diseases, there is now an increasing body of evidence that the intrauterine environment can influence lung growth and development (36). An example of this is the detrimental effect of maternal smoking on fetal lung growth (35, 37). Likewise, the influences of other pre- and perinatal factors, such as premature birth (3, 38), retarded intrauterine growth (16) and neonatal infections (39) seem to increase subsequent respiratory morbidity.

2.4.2. Criticism of the hypothesis

Most studies concerning Barkerˇs hypothesis have been performed in England. In the initial studies, the effects of smoking and alcohol consumption were not taken into account, and at least maternal smoking is known to be associated with increased morbidity in their children (37). Moreover, two other groups of confounding factors, i.e. economical conditions and psychological factors, may, at least partly, explain Barkerˇs findings (40). Several later studies from the year 1992 onwards have not been able to confirm Barkerˇs hypothesis (41-43).

Matthes et al. (41) criticized Barkerˇs hypothesis because no data were available about the lengths of gestations in the Hertfordshire study (4), and a lung function parameter FEV1, instead of the clinical status of the patient, was the main outcome measure. They matched 164
IUGR children (birth weigh < 2500 g at term) with controls (birth weight > 2500 g) at the age of 15 years, and found no differences in clinical diseases or in lung function values, including FEV1, between IUGR children and controls.

Shaheen et al. (42) published a study of 1070 adults, born in Scotland from 1921 to 1935, in whom the recorded information of birth weights and childhood respiratory illnesses were available. In 1985-1986, lung function was measured in 239 (22 %) of these individuals, and there was no association between birth weight, childhood respiratory illnesses and lung function. Only the presence of pneumonia or bronchiolitis before two years age was associated with abnormal FEV1 and FVC values in adults.

Susser and Levin (43) found significant shortcomings in Barker’s epidemiological studies: incomplete samples and follow-ups, inadequate and even missing records, and the absence of comparable data in the controls. These inaccuracies may have predisposed to biased conclusions (44).
2.5. Lung function

Lung function measurements are an objective way to evaluate pulmonary diseases and respiratory capacity. Expiratory and inspiratory flows, reflecting the condition of the airways, can be measured by flow volume spirometry (FVS). Since the FVS requires maximal effort and good co-operation from the subjects, its measurement is possible only from the age of 6-7 years onwards. Forced expiratory flow at 50% of forced vital capacity (FEF50) and forced mid-expiratory flow (FEF25-75) represent the airflow mainly in the small airways, but also reflect compliance of lung tissue (45), and peak expiratory flow (PEF) and forced expiratory volume in one second (FEV1) represent airway flow mainly in larger airways, both being highly effort-depending (45). Absolute lung volumes, reflecting the lung tissue, can be measured by whole body plethysmography, by single breath oxygen test and by open circuit nitrogen washout test. Total lung capacity, vital capacity (VC), forced vital capacity (FVC), functional residual capacity (FRC) and closing volume (CV) represent the lung volumes. In the FVS, PEF shows the peak flow during a maximal expiration of longer than 1 second. In contrast, the parameter measured by Wright’s PEF meter (WPEF) reflects the peak of a maximal, shorter expiration, giving higher results than PEF in the FVS.

2.5.1 Prematurity and lung function

Prematurity

The effects of prematurity alone are difficult to separate from the effects of neonatal respiratory disease because the latter develops in relation to prematurity. Adverse respiratory health outcomes, especially wheezing in the early years of life, have been related to very low birth weight (VLBW), and to prematurity-associated respiratory diseases and the neonatal
intensive care that these infants need (46, 47). In addition, prematurity itself appears to have
effects on lung function and further on lung development independently from any actual
neonatal lung disease (14, 15, 48). Many follow-up studies have demonstrated permanent (49)
or transient airway obstruction at school age or even later in survivors after preterm birth (3,
50). Despite this laboratory evidence, few children with a history of preterm birth appear to
suffer clinically significant asthma or airway obstruction (51).

**Neonatal lung disease**

Prematurity-associated non-acute lung diseases are called bronchopulmonary dysplasia (BPD)
and chronic lung disease (CLD). BPD is defined as a need of supplemental oxygen and
evidence of an abnormal chest x-ray at 28 days of life in preterm children (52). CLD is
diagnosed when, in addition to chest x-ray changes, the oxygen dependency continues beyond
36 gestational weeks (53). Even though BPD and CLD are risk factors for later asthma, and
may induce permanent small airway changes, lung function has been surprisingly normal,
 apart from wheezing or infection periods in the survivors (35). On the other hand, recent
studies have indicated that lung function abnormalities, even though clinically silent, may have
significant functional effects, particularly during exercise (54). For example, non-symptomatic
survivors of CLD use a greater proportion of their ventilatory reserve during exercise than do
other children born pretermly (55).

**Interactions between preterm birth and asthma**

Family histories of asthma and BHR increase the risk of childhood wheezing. This additional
risk has been shown also in children with CLD; when one parent had BHR, the risk of
wheezing increased by 1.7-fold, and when both parents had this disease, the risk increased by
a dramatic 17.8-fold (56). Furthermore, a recent study showed that a family history of asthma
was associated with the presence of CLD (57), and these two factors thus interact together leading to a significant asthma risk in preterm infants. Thus, possibly, there is a genetic predisposition to develop CLD after RDS, or even a genetic predisposition to develop both CLD and asthma (35).

2.5.2 Intrauterine growth retardation and lung function

Prenatal factors

Many factors that have a detrimental effect on fetal growth also have specific effects on lung development; asphyxia, placental insufficiency and cigarette smoke exposure are well-known examples (58). Antenatal corticosteroids have diverse effects. In clinical practice, antenatal corticosteroids have been efficacious in reducing mortality, specifically the incidence of RDS and intracranial haemorrhage in preterm children (59). In addition, the positive effects of antenatal corticosteroids have been additive with surfactant treatment after birth (60). Though increased cortisol secretion due to placental insufficiency theoretically promotes lung maturation, exogenous antenatal steroids impair alveolar development, at least in animal models (27).

In a British cohort study (4), lung functions were studied in 845 men at the mean age of 64 years, and FEV1 fell progressively with decreasing birth weight. In contrast, FVC had no association with birth weight. In another British cohort, 239 adults were followed-up for 60 to 70 years, but no such relationship between birth weight and adult lung function was observed (42). In an Indian cohort, FEV1 and FVC were measured at the mean age of 46 years, with both parameters being subnormal in earlier IUGR children (61).
Threshold time

Stick proposed a threshold time after which airway development is less likely to be affected (35). This time is difficult to assess, since there is an increased risk of respiratory morbidity and an increased need to use therapeutic interventions in relation to prematurity. Both neonatal lung illnesses and their treatment are capable of hindering the normal pattern of lung development. As an example, FEV1 was lower in BPD survivors than in other low birth weight infants when measured at eight years of age (51).

Intrauterine growth retardation vs prematurity

A cross-sectional study in over 5000 children aged 5 – 11 years indicated that lung function was significantly affected by in utero growth whereas respiratory illnesses, especially wheezing, were more related to prematurity (16). The results were confirmed in a recent large epidemiological study (9), in which the effects of prematurity and intrauterine growth, were evaluated both separately and combined in 2470 children aged 5-14 years. In that study, the prevalence of clinical asthma tended to be increased in LBW children. In addition, if the children had required ventilatory support during the neonatal period, FEV1 was lower than in non-ventilated children. The presented data suggest that the effect of intrauterine growth on lung function is more evident than the effect of prematurity.

2.5.3. Respiratory infections and lung function

Lower respiratory infections in infancy

Lower respiratory infections in early childhood have a major confounding effect in studies on intrauterine growth and later development of asthma. Thus, there are many problems encountered in interpreting cross-sectional or retrospective studies. The other difficulty is the
lack of information concerning lung function before any infection. It has been suggested that
subnormal lung function before any illness is a major predisposing factor to wheezing or
pneumonia before the age of three years (24, 62).

_Chand儿hood respiratory illnesses and respiratory morbidity in adults_

A strong positive correlation was found between respiratory mortality in adults and the
presence of bronchitis and pneumonia in infancy also in the original studies of Barker (6). In a
later study, lung function was measured and questionnaire data were collected from over 5000
adult men (4). According to Barker’s hypothesis, a low birth weight was associated with
subnormal adult lung function. In addition, the history of bronchitis, pneumonia, and
whooping cough in infancy further reduced adult lung function. The authors speculated that
the same factors that reduced weight gain _in utero_ also reduced fetal lung growth, and
perhaps, these poorly developed lungs were more prone to succumb to the effects of early
childhood infections.

### 2.6 Bronchial hyperresponsiveness

BHR is an important pathophysiological characteristic of bronchial asthma. The stimuli used
in the challenge tests for BHR, can be subdivided into direct and indirect stimuli (63). Direct
stimuli cause airflow limitation probably by a direct action on the effector cells, such as airway
smooth muscle cells, endothelial vascular cells and mucus producing cells. Indirect stimuli,
instead, probably cause airflow limitation by some action on inflammatory or neuronal cells,
which then interact with the effector cells (64). Examples of directly stimulating agents are
methacholine, histamine, acetylcholine, carbachol, and some prostaglandins and leukotrienes.
Indirect stimuli include physical stimuli such as cold air and dry air, hyperosmolar stimuli like
saline and mannitol, and certain chemical agents, such as adenosine monophosphate, brady-
and tachykinins.

In the European Community respiratory health survey, BHR was measured by the MIC test
and varied from 3 % to 27 % in non-selected child populations of different countries (65).
When the cold air challenge and free running tests have been applied, the respective BHR
frequencies have varied from 13 % to 21 % (66, 67) and from 3 % to 5 % (47, 68),
respectively. Comparison of different studies is difficult due to the differences in study
materials, provocative agents, and in the ways chosen to administer these agents.

2.6.1. Prematurity and bronchial hyperresponsiveness

Increased airway responsiveness in early life is thought to result from complex interactions
between genetic susceptibility (69), and in utero and early-life exposure to environmental
factors (70). Palmer et al. found that BHR, when assessed by histamine challenge at the age of
one month, can predict asthma, atopy, and the degree of BHR at the age of 6 years, implying
that bronchial reactivity has, at least partly, been determined at or before birth (23).
Accordingly, an increased prevalence of BHR, when measured at school age, has been rather
consistently found in prematurely born schoolchildren, irrespective of the presence of RDS
when the child was a newborn (15, 71-73).

Many authors have been unable to confirm the possible relationship between BHR and
neonatal respiratory illnesses or their treatment, or between BHR and other perinatal risk
factors (15, 73, 74). Chan et al (74) found that BHR was related to airway caliber, and
suggested that BHR is a consequence rather than a cause of airway dysfunction.
In addition, von Mutius et al (75) did not find any association between prematurity and BHR in a large epidemiological study in 1993. They studied over 5000 non-selected from 9 to 11 years old children, and found that the children born at < 37 gestational weeks and with birth weight < 2500g did not have increased bronchial reactivity to cold air.

2.6.2. Intrauterine growth retardation and bronchial hyperresponsiveness

Very few studies have evaluated the effects of IUGR on bronchial reactivity. In the study of Wjst et al (9) IUGR was linked to BHR at school age, but only in term children. The authors speculated that the lungs recover better in preterm IUGR children than in term IUGR children.

2.6.3. Respiratory infections and bronchial hyperresponsiveness

Viral respiratory infections can cause BHR in humans, and this observation has been experimentally confirmed in animal models (76). Airway responsiveness usually begins in early life during an acute viral infection, and BHR has been later observed in their responses to histamine, methacholine or citric acid inhalation, or to allergen exposure (77, 78). Acute viral bronchiolitis is the typical infection leading to subsequent BHR (79). In addition, BHR may develop due to other early-life infections or other respiratory insults (80).

It seems that host factors can modify the effects of viral infections on lower airway function. For example, when a viral infection induces BHR, allergic individuals experience greater changes than nonallergic controls (81, 82). Furthermore, subjects having lower FEV1 before the infection, experience greater changes during infection in their airway responsiveness (82).
2.6.4. Consistency of bronchial reactivity throughout puberty

Virtually all infants are hyperresponsive to a variety of stimuli and, rather than being a specific “defect”, the underlying cause is essentially developmental, in particular, attributable to their small airway calibers (24). BHR can develop at any age (but especially during early childhood) as a response to respiratory viral infection, and is believed to result from a damage to airway tissue secondary to local expression of host antiviral immunity (70, 83).

After infancy, the prevalence of BHR follows an U-shape curve: healthy subjects aged less than 13 years and more than 60 years have higher BHR than those aged 13-60 (84). Peat et al found that the prevalence of BHR was high at 7-9 years of age (up to 18 %), decreased significantly at 11-14 years (7-8 %), and then increased in adults (12-14 %) (85). When BHR is repeatedly studied in the same subjects, a declining prevalence of BHR is found: from 20-30 % at the age of 9 years to 10-13 % at the age of 15 years (86).

2.6.5. Challenge tests

Challenge tests are used in the diagnosis of asthma when more simple methods, like spirometry performed before and after administration of a bronchodilator, have not confirmed the diagnosis. In addition, challenge tests can be used to evaluate the degree of bronchial reactivity in clinical work and the frequency of BHR in epidemiological studies.

In both clinical practice and clinical studies, the most often used stimuli in challenge tests have been methacholine, histamine, exercise and cold air (87). The exercise test performed by free running outdoors seems to be especially suitable for epidemiologic studies (68, 88, 89).
Methacholine challenge test

Many different dosing protocols have been used in methacholine challenges (90). The American Thoracic Society has recommended two dosing protocols: (1) the two-minute tidal breathing method and (2) the five-breath dosimeter method allowing the calculation of the cumulative dose of methacholine. The FDA approval for methacholine was based on the five-breath technique and a dosing schedule using methacholine concentrations of 0.025, 0.25, 2.5, 10, and 25 mg/ml. The European Respiratory Society (ERS) has also approved this protocol (87). The two proposed techniques appear to give similar results in both children and adults despite the substantial differences in administration techniques and equipment (91).

The cumulative provocative dose (PD20) (or alternatively the provocative concentration [PC20]), corresponds to the dose triggering a 20 % fall in FEV1 or PEF values. The PD20 values considered to indicate BHR have varied from 400 µg to 4900 µg (92, 93), with 1000 µg or 2000 µg being the most often used thresholds (65). The respective PC20 values have varied between 8-16 mg/ml (90).

Exercise challenge test

The major factors that determine the severity of exercise induced bronchoconstriction (EIB) are the level of pulmonary ventilation reached and sustained during exercise, and the water content and the temperature of the inspired air (90). EIB is probably induced by an abnormally high rate of water loss from the airways (64), leading to a transient hyperosmolarity of the periciliary fluid (the osmolarity hypothesis). Hyperosmolarity of the airway lining fluid is thought to induce a release of inflammatory mediators from epithelial cells (94). According to another theory (the vascular hypothesis) water loss leads to cooling of the airways and in
response to this, the bronchial circulation constricts during exercise. Then, after exercise, a reactive hyperemia follows, leading to airway oedema and obstruction (95).

There are many ways to undertake the exercise for example, a treadmill with adjustable speed, a cycle ergometer with adjustable resistance, and free running indoors or outdoors. The free running test (FRT) outdoors has been proposed as being useful for screening purposes (96), although validity measures and standardizations are difficult to achieve. On the other hand, free running outdoors is a part of the normal life of children, and hyperventilation of cold and dry air mimics a situation that occurs naturally in northern countries.

A fall in FEV1 of as little as 10% seems to be a reasonable criterion for BHR after exercise, because healthy subjects generally demonstrate an increase in FEV1 after exercise (90). Other thresholds used for pathological FEV1 have varied from 8.2% (97) to 15% (98).

*Cold air challenge test*

The isocapnic hyperventilation of cold air (IHCA) test stems from the finding that hyperventilation is the main mechanism behind exercise induced bronchoconstriction (99). Hyperventilation and cold air burden the capacity of the lower airways to humidify and warm the incoming air, and the IHCA test reproduces the symptoms produced by exercise (64). The IHCA test is a rather new method in the diagnosis and monitoring of pediatric asthma (100, 101), and represents a more refined version of exercise testing, because the same pathophysiological mechanism can probably induce bronchospasm.
The most common way of performing the IHCA test is the single-step IHCA: cold dry air is hyperventilated, and CO2 is added to the inspired air to keep the subject eucapnic (100). FEV1 is the monitored parameter, and a fall of 9 % or more from baseline defines BHR (66).

**Comparison of different challenges**

Most subjects with current asthma symptoms have had an earlier evidence of BHR; with prevalence figures ranging from 61 % to 95 % for MIC reactivity (102, 103), from 70 % to 80 % for exercise reactivity (64), and up to 92 % for IHCA reactivity (67).

The IHCA test has been insensitive but very specific for identifying children with asthma (102, 104). In a large epidemiological study, its sensitivity was only 31 %, but its specificity was as high as 88 % to detect "doctor-diagnosed" asthma (66) (Table 1). In contrast, the MIC is a sensitive (75-98 %) but less specific (63-97 %) indicator of BHR (93, 105). Exercise challenge is less sensitive than the MIC test, but like the IHCA test, it is rather specific for separating asthmatic and normal subjects (98, 106, 107). Conducting exercise challenge while breathing cold dry air may be useful if the symptoms are specifically associated with exercise in a cold environment (90). There are no conclusive data comparing the indoor and outdoor exercise tests.
**Table 1.** Comparison of different challenge tests for diagnosing bronchial hyperresponsiveness.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Subjects</th>
<th>Age (years)</th>
<th>Challenge</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>BHR criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hopp(^a)</td>
<td>1984</td>
<td>165</td>
<td>5-21</td>
<td>Methacholine</td>
<td>98</td>
<td>63</td>
<td>PD20(^c) &lt; 800 breath units</td>
</tr>
<tr>
<td>Pöysä(^a)</td>
<td>1992</td>
<td>120</td>
<td>9-10</td>
<td>Methacholine</td>
<td>75</td>
<td>97</td>
<td>PD20(^d) &lt; 4900 μg</td>
</tr>
<tr>
<td>Remes(^a)</td>
<td>2002</td>
<td>218</td>
<td>7-13</td>
<td>Methacholine</td>
<td>47</td>
<td>97</td>
<td>PD20(^d) ≤ 400 μg</td>
</tr>
<tr>
<td>Weiss(^a)</td>
<td>1984</td>
<td>213</td>
<td>5-24</td>
<td>Cold air</td>
<td>43</td>
<td>81</td>
<td>FEV% change &gt; 9 %</td>
</tr>
<tr>
<td>Nicolai(^a)</td>
<td>1993</td>
<td>5697</td>
<td>9-11</td>
<td>Cold air</td>
<td>31</td>
<td>88</td>
<td>FEV1 change &gt; 9 %</td>
</tr>
<tr>
<td>Jones(^b)</td>
<td>1994</td>
<td>956</td>
<td>4-11</td>
<td>Exercise</td>
<td>58</td>
<td>97</td>
<td>PEF change &gt;15 %</td>
</tr>
<tr>
<td>Haby(^a)</td>
<td>1994</td>
<td>96</td>
<td>8-11</td>
<td>Exercise</td>
<td>27</td>
<td>95</td>
<td>FEV1 change &gt;13 %</td>
</tr>
<tr>
<td>Remes(^a)</td>
<td>2002</td>
<td>218</td>
<td>7-13</td>
<td>Exercise</td>
<td>35</td>
<td>97</td>
<td>FEV1 change &gt; 10 %</td>
</tr>
</tbody>
</table>

\(^a\) Patients with asthma and controls

\(^b\) Patients with and without asthma 6 years later

\(^c\) The provocative dose of methacholine causing a 20 % fall in FEV1 or \(^d\) WPEF

Though different subjects respond to different stimuli in the challenge tests (108), most studies have shown a strong correlation between the responses to cold air and to either histamine or methacholine (109).
Given the high variability of BHR measurements in relation to time, a single BHR test may not give reliable information about the responsiveness of the airways or about the tendency to subsequently develop asthma. Burrows et al showed that repeated positive MIC results predicted almost 85% of the later wheezing patients (86).

*BHR in non-symptomatic children*

There is increasing evidence that BHR in non-symptomatic children is a risk factor for the development of asthma or asthma-like symptoms in later life (110). Although only 14% of asymptomatic adults with BHR in the MIC test developed asthma during a 3-year follow-up (111), the risk for asthma increased when the follow-up was longer; the figure was 50% in a study with a 17-year follow-up (112). In children, the figures have been even higher. Peat et al followed 236 children aged 8 to 11 years over a period of 12 months (113), and 52% of the initially asymptomatic BHR positive children became wheezers. Jones et al studied 55 asymptomatic BHR positive and 55 asymptomatic BHR negative children, aged 5 to 11 year (106). Six years later, 58% of the subjects in the BHR positive group had developed asthma. Carey et al followed-up 281 adolescents over a period of 18 months, and found that BHR was significantly and independently of other factors associated with asthma (114).

2.7. Asthma and bronchial hyperresponsiveness in twin studies

Räsänen et al evaluated over 6000 adolescent twins in a Finnish nationwide population-based study (115). They found an increasing risk for asthma with increasing ponderal index (kg/m³) at birth, that is with increasing relative birth weight. However, when adjusted for gestational age, the birth weight lost its significance. Asthma was present in 32 persons among the 1130 discordant monozygotic pairs, and the twins with asthma tended to have a lower birth weight and
lower ponderal index than their unaffected co-twins. Their conclusions were based on questionnaire data only, and no clinical or lung function data were available. They suggested that twins are more prone to the negative pulmonary effects caused by IUGR than singletons. Therefore, the harmful effects of IUGR on lung function found in twin studies may not be generalizable to all children. Clarke et al studied nearly 400 from 8 to 18 years old twins and found that the associations with asthma, atopy and BHR were greater in monozygotic than in dizygotic twin pairs (116). They did not correlate asthma and BHR with birth weight differences. Thus, the observations of Räsänen et al (115) are thus far the only available data correlating intrauterine growth with asthma in twins. There is a complete paucity of corresponding data available about lung function and IUGR.
3. AIMS OF THE STUDY

The main purpose of the study was to investigate, whether the prenatal growth pattern associates with lung function and bronchial reactivity at school age and early adolescence, and whether the changes, if present, remain consistent through puberty.

The specific aims were:

1. To investigate the effects of IUGR on expiratory flows in spirometry measured at school age. The spirometer data were supplemented for lung volumes measured by a single breath oxygen test and by an open circuit nitrogen washout test (I).

2. To evaluate the effects of IUGR on bronchial responsiveness, as measured by cold air and methacholine challenge tests at the same age (II, III).

3. To evaluate the consistency of cold air reactivity during a six-year follow-up period (II, unpublished follow-up data).

4. To compare how well the bronchial challenge tests with a direct (methacholine) and indirect (cold air) stimulus, supplemented by the free running test, identify patients with BHR (IV).
4. MATERIAL AND METHODS

4.1. Subjects

Studies I, II and III. The selection and numbers of study subjects are shown in Figure 1. The final study group consisted of 67 children: 30 IUGR children and 37 AGA children. The neonatal morbidity of these 67 children is presented in Table 1/I. One child had mild cerebral palsy, and this pair was excluded from the study. No other pairs were excluded due to underlying illnesses.

Figure 1. The selection of children and the study design
Study IV. The PD20 (provocative dose inducing a 20% or more fall in WPEF) in the MIC test was < 4900 µg in 23 children, including five twin pairs with PD20 < 4900 µg in both children (III). These 23 reactive children and their 13 non-reactive counterparts were requested to perform the exercise test. Twenty-nine children finally attended the test, and they form the study group examined in study IV.

Patients re-studied at adolescence (unpublished data). In the survey in 1993, the IHCA challenge test was carried out in 63 children (II). A follow-up study was performed in 1999, and 53 (84%) subjects re-performed the IHCA test using the same method as 6 years earlier.

4.2. Questionnaire data

Study I. The parents completed the questionnaire regarding the health problems of their children after neonatal hospital stay until the time of examination in year 1993.

Studies II and III. The reliability of the respiratory history obtained from the questionnaire was confirmed by checking the hospital and health center medical cards of the children prior to the examination. The numbers of respiratory infections, antibiotic courses and episodes of acute otitis media (only those cases diagnosed by a doctor were included), and procedures like adenoidectomy, tonsillectomy and tympanostomy were recorded. If one or more of these procedures had been done, the subjects were included into the "ENT (Ear Nose Throat) surgery group".

Follow-up study (unpublished data). The parents and children completed the "Tuohilampi" questionnaire, specifically designed for assessing asthma and asthma-related symptoms covering the period from the first study until the follow-up study in 1999 (117).
4.3. Intrauterine growth analysis

Based on growth status at birth, infants were sub-divided into appropriate grown (AGA) and intrauterine growth retarded (IUGR) groups. For that purpose, birth weights were transformed to gestational age-specific SD units by using fetal growth charts for Finnish newborn children, available separately for girls and boys (118). AGA was defined as the birth weight ranging from -2 to +2 SD units. IUGR was diagnosed if the gender- and gestational age-specific birth weight was < -2 SD units or if the within-pair birth weight difference was > 1.3 SD units. This twin-pair birth weight difference was the border of the lower quartile (25th percentile) of all twin-pair birth weight differences. For more details, see Table 2/I.

4.4. Lung function and challenge tests at school age

4.4.1. Lung functions (I, II, III, IV)

Lung functions were examined by the 2200 Computerized Pulmonary Function Laboratory from Sensor Medics (USA). Lung functions included: 1) lung subdivisions (slow vital capacity, VC); 2) forced expiratory flow rates PEF (peak expiratory flow), FEV1 (forced expiratory volume in one second), FEF50 (forced expiratory flow at 50 % of the maximal VC), FEF75 (forced expiratory flow at 25 % of the maximal VC and FEF25-75 (forced mid-expiratory flow) by flow-volume spirometry (FVS); 3) the single breath oxygen test (closing volume, CV); 4) the open circuit nitrogen washout test (functional residual capacity, FRC). Among these parameters, VC, FRC and CV represent lung volumes. The parameters assessed in the FVS represent airway flow values.
The lung functions were measured according to the recommendations of the American Thoracic Society (119). Lung function data were standardized using height-related reference values; the references were separate for boys and girls, and were expressed as percentages of predicted values (% predicted) (120). Only the FEF25-75 reference equation was based on combined data from both sexes (121). The lower limits for normality were 75 % for PEF, FEV1 and FEV% (FEV1/forced vital capacity ratio), and 65 % for FEF50 and FEF25-75 (122). PEF was also measured by Wright’s peak flow meter (WPEF). The lower limit of normality was 75 % for WPEF (122).

4.4.2. Cold air challenge test (II and the follow-up study)

The IHCA challenge was carried out in a warm room with the method originally reported by Zach (100), and modified by Koskela (123). Before challenge, three technically acceptable baseline flow-volume curves were obtained using a pneumotachograph spirometer (Medikro 909; Medikro Ltd; Kuopio, Finland), while the subjects were sitting in room temperature at normal humidity. By using the highest FEV1 value, the target minute ventilation was set to FEV1 x 25. Carbon dioxide (CO2) was added to the inspired air, and the flow was calculated as the target minute ventilation x 0.05. Air containing water less than 1.75 mg/L was cooled in the heat exchanger (Jaeger RHES; Erich Jaeger GmbH & CoKG; Germany) and released through a mouthpiece. At the inspiratory port of the mouthpiece, 8 cm from the mouth, the temperature of the inspired air was continuously monitored (GTH 1200 Digital thermometer; Greisinger Electronic), and the aim was to keep the inspired air temperature in the range of -12 °C to -15 °C during hyperventilation. The duration of hyperventilation was 4 minutes. At least 3 technically satisfactory maximal FVS curves were obtained at 3, 5, and 10 minutes
after the end of hyperventilation. The best of the three FEV1 values was included in further analyses.

The maximal percentage fall in FEV1, calculated from the highest pre-challenge and the lowest technically acceptable post-challenge FEV1, was used to express the response, and a fall of 9% or more was considered as pathological (67, 99, 100, 123). The subjects with an abnormal IHCA test results were termed as cold responders.

4.4.3. Methacholine challenge test (III)

The MIC test was based on a multiple breath technique and a step-by-step increasing dosing schedule using a methacholine concentration of 2.5 mg/ml with one, three and eleven inhalations, and thereafter, the concentration of 25 mg/ml with three, ten and ten inhalations. The methacholine solution was administered by a Spira Electro 2 dosimeter (Respiratory Care Center, Hämeenlinna, Finland). The volume of each inhalation and the number of nebulizations are displayed as digital readouts; thus, the total amount of methacholine inhaled by the patient can be calculated (93, 124). Before each MIC testing, three separate WPEF values were measured, and the best value was recorded for further analyses. The cumulative provocative dose causing a 20% fall in WPEF (PD20) is the extrapolated cumulative dose of methacholine which has been inhaled before the WPEF fell 20%. Inspired methacholine amounts were summed; an increased responsiveness was considered to be present if PD20 was below 1000 μg, and those subjects were termed as methacholine responders. The maximum cumulative dose of methacholine was 4900 μg; when this dose was reached the test was stopped.
Changes in PEF often parallel changes in FEV1 during bronchoconstriction but have the disadvantage of being more effort dependent, less reproducible and less sensitive in detecting bronchoconstriction (125). Thus the change in FEV1 has become the recommended primary outcome measure for the MIC test in recent protocols (90). WPEF was used in this present study, because it was the routinely used method in our hospital and in our previous studies (93).

4.4.4. Exercise challenge test (IV)

Eight minutes of outdoor free running at a heart rate > 170/min (about 85% of maximum) constituted the exercise challenge, and it was performed during cold seasons, from August 1993 to March 1994, in accordance with the protocol of Haby et al (98, 126). Heart rate was monitored by telemetry (Polar Sport tester, Polar Elektro Ltd., Kempele, Finland) at 15 seconds intervals. Both WPEF and FEV1 values were monitored, and the best of the 3 blows was recorded. WPEF was measured before and immediately after the test, and 5, 10, 15, and 20 min later. Likewise, FEV1 was measured by the dynamic spirometry (Vitalograph dry spirometer, Hamburg, Germany) before and immediately after the test, and 10 and 20 min later. The lungs of the subjects were auscultated before, and 5 and 15 minutes after the exercise. Symptoms and signs if present, and possible auscultatory findings were recorded. The median outdoor temperature during the exercise was -1°C (range -12°C to +10°C). A positive exercise test result was defined as a 15% or greater fall in WPEF or FEV1 at any time after the exercise, calculated from the baseline pre-exercise values (126).
**Bronchodilation test**

Twenty-five minutes after exercise all children received salbutamol 0.15 mg/kg via Spira nebulizer (Spira Oy, Hämeenlinna, Finland). WPEF and FEV1 were measured 15 min after bronchodilator inhalation. A positive bronchodilation test result was defined as a 15 % or greater rise in WPEF or FEV1 after bronchodilator inhalation calculated from the baseline pre-exercise values (127).

In the present study, children with a positive response in the exercise test were termed as exercise responders, and children positive in the bronchodilation test as bronchodilator responders. The children with a positive response either in the exercise test or in the bronchodilation test were called the exercise-bronchodilation responders.

**4.5. Lung function and challenge tests in adolescence (unpublished data)**

The baseline lung functions of all the 53 children attending the follow-up study were measured in the same ways as in Study I. In addition, a pneumotachograph flow-volume spirometer (Medikro 909; Medikro Ltd; Kuopio, Finland) was used for both baseline lung functions and FEV1 monitoring in the IHCA test. The respective results obtained by the same pneumotachograph spirometer were available also from the year 1993, and these results were compared with the year 1999 results.
4.6. Statistical methods

Statistical analyses were performed by using SPSS for Windows 6.1.2-4 (Chicago, Illinois).

**Study I.** Spearman’s correlation coefficient was used to assess the correlations between standardized birth weight and gestational age and lung function parameters.

Intra-pair correlations were calculated by using the Wilcoxon matched-pairs signed ranks test. The t-test for independent samples was used in calculating differences in pulmonary functions between IUGR and AGA groups.

**Study II.** Comparisons of differences in categorical variables between the IHCA responders and non-responders were tested with the chi-square independence test. The significances of interval scale and continuous variables between the IHCA responders and non-responders and between the IUGR and AGA groups were evaluated using the t-test for normally distributed variables and the Mann-Whitney U-test for non-normally distributed variables. Intra-pair differences in the IHCA test were calculated by the Wilcoxon matched-pairs signed rank test.

**Study III.** The Mann-Whitney U-test was used for continuous variables and the Fischer’s exact test for non-continuous variables to examine differences between IUGR and AGA children and between MIC responders and non-responders. Intra-pair differences in the MCT test were examined by using the Wilcoxon matched-pairs signed ranks test. The McNemar test was applied to evaluate the association between BHR and IUGR within discordant twin pairs.

Cohen’s kappa values were calculated to evaluate the agreement between WPEF and FEV1 changes in the MIC test.

**Study IV.** The Mann-Whitney U test was used for non-normally distributed continuous variables and the t-test for normally distributed continuous variables to examine the differences between IUGR and AGA children and between cold, methacholine, exercise or bronchodilation responders and non-responders. The non-continuous variables were tested with Fischer’s exact
test. The McNemar test was applied to evaluate the association between BHR and IUGR within discordant twin pairs. The Pearson correlation coefficients between baseline FEV1 and WPEF, as well as between exercise induced falls of FEV1 and WPEF were calculated. Cohen's kappa values were calculated to evaluate the agreement between the results yielded by different bronchial challenges.

The follow-up study. The Mann-Whitney U test was used for non-normally distributed continuous variables to examine the differences between IUGR and AGA children in baseline expiratory flow values and in cold responses. The non-continuous variables were tested with the Fischer's exact test. The Wilcoxon matched-pairs signed ranks test was used in paired expiratory flow comparisons of the same children six years apart, and in lung function or challenge comparisons within the 19 disproportionally grown pairs. Repeated measures ANOVA (analysis of variance) was used for analyzing expiratory flow values of the 19 disproportionally grown pairs between the two surveys six years apart.

4.7. Ethics

The parents of the children (studies I-IV) provided oral informed consent for the participation in the study. A written consent was provided from the adolescents for the participation in the follow-up study. The study was approved by the Research Ethics Committee of Kuopio University Hospital.
5. RESULTS

5.1. Patient characteristics and baseline lung functions (I)

The basic characteristics of the 67 study subjects are presented in Table 2. The distributions of the birth measures and gestational ages in IUGR and AGA children are given in Table 3.

Table 2. Patient characteristics during the neonatal period in the 67 study subjects

<table>
<thead>
<tr>
<th>Study children</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls/Boys</td>
<td>35/32 (52/48 %)</td>
</tr>
<tr>
<td>Apgar score(^1) 1 min / 5 min</td>
<td>8 (1-9) / 9 (5-10)</td>
</tr>
<tr>
<td>Ventilator treatment</td>
<td>21/67 (32 %)</td>
</tr>
<tr>
<td>Duration of ventilator treatment(^1) (days)</td>
<td>&lt; 1 (0-32)</td>
</tr>
<tr>
<td>Duration of hospital stay(^1) (days)</td>
<td>22 (4-154)</td>
</tr>
<tr>
<td>Duration of supplementary oxygen(^1) (days)</td>
<td>2 (&lt;1-138)</td>
</tr>
<tr>
<td>RDS(^2)</td>
<td>12/67 (18 %)</td>
</tr>
<tr>
<td>CLD(^3)</td>
<td>2/67 (3 %)</td>
</tr>
<tr>
<td>IUGR/AGA</td>
<td>30/37 (45/55 %)</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>5/67 (8 %)</td>
</tr>
<tr>
<td>PDA(^4)</td>
<td>2/67 (3 %)</td>
</tr>
</tbody>
</table>

\(^1\)Median (range)

\(^2\)Respiratory distress syndrome; homogenous reticulogranular pattern in chest x-ray and need for oxygen in premature infants

\(^3\)Chronic lung disease; need for supplemental oxygen and chest x-ray changes at 36 weeks of corrected postnatal gestational age

\(^4\)Patent ductus arteriosus (clinical diagnosis)

Neonatal morbidity, expressed by different clinical characteristics, were rather similar in the IUGR and AGA children (data not shown).
Table 3. Absolute and relative birth measures and gestational ages in IUGR and AGA children

<table>
<thead>
<tr>
<th>Birth weight and gestational weeks</th>
<th>IUGR</th>
<th>AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>30</td>
<td>37</td>
</tr>
<tr>
<td>Absolute birth weight $^1$ (g)</td>
<td>1895 (800-2490)</td>
<td>2230 (1100-3150)</td>
</tr>
<tr>
<td>Relative birth weight $^1$ (SD $^2$)</td>
<td>- 2.5 (-4.7 - +0.1)</td>
<td>- 0.7 (-1.9 - +1.6)</td>
</tr>
<tr>
<td>Gestational age in weeks $^1$</td>
<td>35 (28-38)</td>
<td>34 (28-38)</td>
</tr>
</tbody>
</table>

$^1$ Median (range).

$^2$ The relative birth weight is expressed as SD units based on gender- and gestational age-specific reference values.

The length, weight, atopy and smoking data in the 67 study subjects at the time of lung function examinations are expressed in Table 4. These factors were similar in the IUGR and AGA groups.
Table 4. Data on length, weight, presence of atopic diseases and smoking habits in study subjects.

<table>
<thead>
<tr>
<th></th>
<th>IUGR</th>
<th>p</th>
<th>AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length(^1) (cm)</td>
<td>139.5 (120.0-163.9)</td>
<td>ns</td>
<td>139.0 (125.0-181.0)</td>
</tr>
<tr>
<td>(SD)</td>
<td>-0.4 (-3.0- +0.7)</td>
<td>ns</td>
<td>-0.1 (-3.3- +3.2)</td>
</tr>
<tr>
<td>Weight(^1) (kg)</td>
<td>34.5 (17.8-53.4)</td>
<td>ns</td>
<td>32.8 (19.1-43.0)</td>
</tr>
<tr>
<td>(%)(^2)</td>
<td>-1.5 (-21- +46)</td>
<td>ns</td>
<td>0 (-22- +27)</td>
</tr>
<tr>
<td>Atopy(^3)</td>
<td>7/30</td>
<td>ns</td>
<td>6/37</td>
</tr>
</tbody>
</table>

Smoking

- In year 1993: 4/30 ns 2/37
- In year 1999: 7/23 ns 6/30

Length and weight data are presented from the year 1993, smoking data from both 1993 and 1999.

\(^1\)Median (range)

\(^2\)Deviation of weight from the mean weight for height

\(^3\)Atopic dermatitis, allergic rhinitis and/or allergic conjunctivitis

The expiratory flow values and lung volumes in IUGR and AGA children are expressed in Figure 2. The lung volume parameters were within the normal reference ranges in all subjects in both IUGR and AGA groups. The lung volumes did not associate with gestational age or with gestational age adjusted birth weight. Baseline FEF50 values were lower in the IUGR than in the AGA children. Accordingly, within discordant twin pairs, the IUGR twins had significantly lower FEF50 values than their AGA counterparts (Fig 3/I). No other significant differences in expiratory flows were observed between these intrauterine growth groups. Five (22 \%) of the IUGR and four (13 \%) of the AGA children had at least one abnormal FVS result (ns).
Figure 2. The median (75th percentile) expiratory flows in the 30 IUGR and 37 AGA children. The numbers of children with abnormal values are expressed inside the columns. * p=0.04 vs AGA children. For the definition of abnormal results, see the text.

5.2. Responsiveness to cold air (II)

Sixteen (25 %) of the 63 children had a greater than 9 % fall in FEV1 in the IHCA test (Figure 3). Eight (30 %) of the 27 IUGR children were cold air responders; the respective figure was 8 (22 %) in the 36 AGA children (ns). The median FEV1 fall in these 16 responders was 10 %, with the greatest FEV1 fall being 24 %. In the 47 non-responders, in fact, FEV1 fell in 38 cases, the median fall being 3.5 %
Figure 3. The changes in FEV1 values in the IHCA test. The maximal fall was calculated using the highest pre-challenge and lowest post-challenge FEV1, and a fall of 9% or more was considered as pathological. The shaded parts of the columns represent IUGR children.

The perinatal or neonatal factors did not differ between the cold air responders and non-responders (Table 2/II). Invasive ENT (Ear-nose-throat) procedures, such as adenoidectomy, had been done significantly more often, 56% vs 23% (p<0.05), in the cold air responders than in the non-responders (Table 4/II). On the other hand, antibiotic courses or physician visits for infections did not differ between the groups. The intrauterine growth pattern was not connected to any of the respiratory infection parameters (data not shown).

The cold responders had significantly lower baseline FEV%, FEF50 and FEF25-75 values than the non-responders (Table 5). In all, 16% of the cold responders and 14% of the non-responders had subnormal baseline expiratory flows.
5.3. Responsiveness to methacholine (III)

Ten (15\%) of the 67 children had an abnormal response (PD20 < 1000 \(\mu\)g) in the MIC test (MIC responders). Forty-four children (66\%) had no responses at all (PD20 > 4900 \(\mu\)g). The distribution of the PD20 values in the study children is presented in Figure 4.

![Figure 4](image)

**Figure 4.** The distribution of PD20 values in the MIC test. PD20 represents the provocative dose of methacholine producing a 20\% fall in WPEF values. The 30 IUGR children are presented separately with shaded bars.

Perinatal and neonatal factors did not differ between the MIC responders and non-responders (data not shown). The MIC test results were not connected to the intrauterine growth pattern. However, 6 (60\%) of the 10 children with PD20 < 1000 \(\mu\)g belonged to the IUGR group. The responders had significantly lower baseline PEF, FEV1, FEF50 and FEF25-75 values than the nonresponders (Table 5). In all, 50\% of the MIC responders but only 7\% of non-responders had subnormal baseline expiratory flows (p<0.05).
Table 5. Baseline expiratory flow rates, presented separately in relation to cold air reactivity and to methacholine reactivity.

<table>
<thead>
<tr>
<th>Spirometry</th>
<th>Cold air challenge</th>
<th>Methacholine challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Responders (n=16)</td>
<td>Non-responders (n=47)</td>
</tr>
<tr>
<td>PEF\textsuperscript{1}</td>
<td>98 (79-120)</td>
<td>96 (54-139)</td>
</tr>
<tr>
<td>FEV1\textsuperscript{1}</td>
<td>93 (83-104)</td>
<td>96 (67-110)</td>
</tr>
<tr>
<td>FEV\textsuperscript{2}%</td>
<td>94 (80-110)*</td>
<td>100 (85-115)</td>
</tr>
<tr>
<td>FEF50\textsuperscript{1}</td>
<td>86 (57-113)*</td>
<td>99 (58-144)</td>
</tr>
<tr>
<td>FEF25-75\textsuperscript{1}</td>
<td>85 (56-115)*</td>
<td>101 (57-152)</td>
</tr>
<tr>
<td>Children with abnormal flow-volume spirometry</td>
<td>3 (16 %)</td>
<td>7 (14 %)</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Median (range)

For abbreviations of PEF, FEV1, FEV\%%, FEF50 and FEF25-75, see the text

\* p<0.05 between responders and nonresponders, presented separately for both challenges

Doctor-diagnosed respiratory infections, numbers of antibiotic courses, episodes of otitis media and the need for ENT surgery were more common in the MIC responders than in the non-responders (Table 5/III). With respect to ENT surgery, the difference between the MIC responders and nonresponders was significant only in the IUGR children. In them, 67 % of MIC responders and 13 % of non-responders belonged to the ENT surgery group (p=0.016)
5.4. Comparison of the challenge tests (IV)

Three challenge tests, that is cold air inhalation, methacholine inhalation and exercise-
bronchodilation test, were all performed in 29 children, and the tests were done on different
days. Eighteen (62%) subjects exhibited one or more positive responses; the figure was 11/14
in the IUGR and 7/15 in the AGA groups (ns). The numbers of responders in the three
challenge tests are illustrated in Figure 5. Ten (34 %) children responded in two tests, and
seven (70%) of them belonged to the IUGR group. In addition, the two children responding in
all three test were IUGR cases.

**Figure 5.** The distributions of the nine methacholine, ten cold air and 11 exercise-
bronchodilation responders between the different bronchial challenge tests. The IUGR
children are expressed as black circles and the AGA children as open circles.

The cold air responses were associated with responses in the exercise and exercise-
bronchodilation test, but not with the methacholine reactivity. The kappa value between the
responders in the cold air and exercise tests was 0.68, and in the cold air and exercise-bronchodilation tests it was 0.48. There was no association between baseline lung function and exercise, bronchodilation or exercise-bronchodilation test results (data not shown).

5.5. Lung function and responsiveness to cold air in adolescence (unpublished data)

The baseline expiratory flow values did not differ between the subjects in the IUGR and AGA groups (Table 6). Abnormalities were rare; FEV1 was in all cases within normal limits, and the figures were under 10 % for FEV% and PEF. The figures were higher for FEF50 and FEF25-75 values, being 13 % - 17 % in the IUGR and 7 % - 10 % in AGA groups (ns). At least one abnormal result was seen in 22 % of the IUGR and in 13 % of the AGA cases (ns).

In the IHCA test, the mean changes in FEV1 values were - 4.7 % in IUGR children and - 4.3 % in AGA children (ns). Among all of the 53 cases, four (18 %) in the IUGR and one (3 %) in the AGA group were IHCA responders (> 9 % decrease in FEV1) (ns).
Table 6. Baseline expiratory flows (% predicted) and proportions of subjects with abnormal values in relation to the intrauterine growth status in the 53 study subjects in 1999

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IUGR (n=23)</th>
<th>AGA (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1</td>
<td>96 (76-112)</td>
<td>92 (77-118)</td>
</tr>
<tr>
<td></td>
<td>0^2</td>
<td>0^2</td>
</tr>
<tr>
<td>FEV%</td>
<td>87 (69-96)</td>
<td>89 (67-100)</td>
</tr>
<tr>
<td></td>
<td>2 (9 %)</td>
<td>1 (3 %)</td>
</tr>
<tr>
<td>PEF</td>
<td>107 (74-152)</td>
<td>101 (72-137)</td>
</tr>
<tr>
<td></td>
<td>2 (9 %)</td>
<td>1 (3 %)</td>
</tr>
<tr>
<td>FEF50</td>
<td>84 (54-127)</td>
<td>84 (53-134)</td>
</tr>
<tr>
<td></td>
<td>4 (17 %)</td>
<td>3 (10 %)</td>
</tr>
<tr>
<td>FEF25-75</td>
<td>93 (55-133)</td>
<td>100 (58-144)</td>
</tr>
<tr>
<td></td>
<td>3 (13 %)</td>
<td>2 (7 %)</td>
</tr>
<tr>
<td>At least one abnormal result</td>
<td>5 (22 %)^2</td>
<td>4 (13 %)</td>
</tr>
</tbody>
</table>

The limits considered as abnormal were < 75 % for FEV%, PEF and FEV1, and < 65 % for FEF50 and FEF25-75

^1 Median (range)

^2 Cases with an abnormal value

There were no significant differences in any parameters between the IUGR and AGA groups.
5.6. Comparison of lung function and cold air challenges between the 1993 and 1999 studies (unpublished data)

As a part of the IHCA test in 1999, lung functions were measured by the pneumotachograph spirometer, and the respective values obtained by the same spirometer were available also from the 1993 study (not used in the original articles). The same spirometer and the certified quality system of the laboratory guarantee that the results are comparable. We analysed the baseline FVS values and the IHCA test responses of the 19 disproportionally grown twin pairs in more detail. The results of the baseline lung function and the IHCA test from the year 1993 are shown in Table 7, and the respective results from the year 1999 in Table 8. In 1993, expiratory flows were not associated with intrauterine growth pattern. In 1999, the IUGR twin pairs had lower FEV% and FEF25-75 values than their AGA counterparts. The cold air-induced FEV1 decrease was rather similar in the smaller and larger twin pairs in both studies. However, in the follow-up study in 1999, all of the four IHCA responders were among the smaller twin pairs.
Table 7. Lung function parameters and cold air challenge results
in disproportionally grown 19 twin pairs in 1993

<table>
<thead>
<tr>
<th>Lung function Parameter</th>
<th>Smaller twin-pair</th>
<th>p</th>
<th>Larger twin-pair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline expiratory flows</td>
<td>FEV1 97 (81-121) $^1$</td>
<td>ns</td>
<td>99 (65-171) $^1$</td>
</tr>
<tr>
<td></td>
<td>FEV% 89 (72-100)</td>
<td>ns</td>
<td>89 (73-97)</td>
</tr>
<tr>
<td></td>
<td>PEF 123 (83-150) $^#$</td>
<td>ns</td>
<td>123 (81-159)</td>
</tr>
<tr>
<td></td>
<td>FEF50 93 (55-125)</td>
<td>ns</td>
<td>97 (57-125)</td>
</tr>
<tr>
<td></td>
<td>FEF25-75 90 (57-128)</td>
<td>ns</td>
<td>99 (51-130)</td>
</tr>
<tr>
<td></td>
<td>One pathological result 2 (11 %)</td>
<td>ns</td>
<td>2 (11 %)</td>
</tr>
<tr>
<td>Baseline lung volumes</td>
<td>FVC 98 (84-133) $^1$</td>
<td>ns</td>
<td>103 (78-120) $^1$</td>
</tr>
</tbody>
</table>

Cold air challenge test

| FEV1 change (%) | ns | 4.7 $^1$ |
| (+12.8- -24.3) | (+6.3- -12.6) |
| Pathological results $^2$ | 5 (26 %) | ns | 4 (21 %) |

$^1$ Median (range)

$^2$ > 9 % decrease in FEV1

$^#$ Statistical significance between the 1993 and 1999 studies: p <0.0001; no other significant differences were present
Table 8. Lung function parameters and cold air challenge results in disproportionally grown 19 twin pairs in 1999

<table>
<thead>
<tr>
<th>Lung function parameter</th>
<th>Smaller twin-pair</th>
<th>p</th>
<th>Larger twin-pair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline expiratory flows</td>
<td>FEV1 99 (76-112)</td>
<td>ns</td>
<td>103 (77-118)</td>
</tr>
<tr>
<td></td>
<td>FEV% 87 (69-96)</td>
<td>0.01</td>
<td>91 (68-100)</td>
</tr>
<tr>
<td></td>
<td>PEF 104 (74-152)</td>
<td>ns</td>
<td>112 (84-137)</td>
</tr>
<tr>
<td></td>
<td>FEF50 84 (51-127)</td>
<td>ns</td>
<td>88 (53-134)</td>
</tr>
<tr>
<td></td>
<td>FEF25-75 93 (55-133)</td>
<td>0.03</td>
<td>102 (58-144)</td>
</tr>
<tr>
<td></td>
<td>One pathological result 5 (26 %)</td>
<td>ns</td>
<td>2 (11 %)</td>
</tr>
<tr>
<td>Baseline lung volumes</td>
<td>FVC 95 (77-149)</td>
<td>ns</td>
<td>108 (84-138)</td>
</tr>
<tr>
<td>Cold air challenge test</td>
<td>FEV1 change - 3.6</td>
<td>ns</td>
<td>- 4.8</td>
</tr>
<tr>
<td></td>
<td>(% (+8.7- -19.6))</td>
<td></td>
<td>(+1.3- -8.8)</td>
</tr>
<tr>
<td></td>
<td>Pathological results 4 (21 %)</td>
<td>0.04</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Median (range)
2 > 9 % decrease in FEV1
3 See Table 6

The 6-year time between lung function tests had a significant effect only on PEF results; PEF values decreased during the follow-up, but significantly only in the IUGR pairs. In all of the other parameters, the changes were minor and seemed to be arbitrary.

Nine children exhibited a pathological IHCA result in 1993. The figure decreased to four in 1999; two of them had been and two had not been cold responders six year earlier. Thus, responsiveness to cold improved in seven subjects but continued in two others through puberty. Both children who continued to have BHR to cold air had suffered from wheezing between the ages of 7 and 18 years. These two study subjects, a 15-year old boy and an 18-year old girl, probably were asthmatics.
6. DISCUSSION

6.1. Study design

In this thesis, respiratory morbidity, lung function and bronchial responsiveness were studied two times six years apart, in 67 subjects at school age and in 53 subjects in adolescence. The children were from multiple pregnancies sharing the same intrauterine environment, they were born prematurely, and they had had only minor pulmonary disability during the neonatal period. Both gestational age and birth weight were taken into account in the analyses. This study design minimises genetic and environmental confounding factors, and thus allows for the evaluation of the effects of intrauterine growth to later health. Moreover, the follow-up time was long, ranging from 13 to 21 years.

The children were studied clinically, by conventional spirometry, by two tests for lung volumes and by three different bronchial challenges. The challenges were done on different days, and the bronchodilation test was done on the last day of the study. Since we performed the FVS and cold air challenge by identical methods six years apart, the consistency of expiratory flows and BHR could be evaluated.

The evaluation of lung volumes in the second survey was not optimal, since only FVC, not absolute lung volumes, was measured. Therefore, it was not possible to assess the consistency of lung volumes.
For the review of the literature, more than 100 studies were checked for birth weight and later respiratory outcome. There were only three published studies, in which the birth weight had been either adjusted for gestational age as in the present thesis (9, 16), or had been taken into account in some other way (47). For comparison with the findings of the present study, the study designs and main results of these three studies are presented in Table 9. Two studies were large epidemiological studies (9, 16), one was a clinical case-control study (47).

The study of Rona et al (16) evaluated the effects of birth weight and prematurity on lung function and respiratory illness in over 5500 five to 11 years old children living in the UK. The lung functions and respiratory symptoms were expressed in relation to gestational age and to gestational age adjusted birth weight. IUGR was not clearly defined, but the available data indicated that there were at least 122 term IUGR children. In all, 727 children were born prematurely, but no information about their intrauterine growth was presented.

The study of McLeod et al (47) evaluated respiratory health and lung function between 300 VLBW children whose birth weight was < 1500 g, and classroom controls with normal birth weights, at the age of 8 - 9 years. Of these 300 VLBW children, 94 were IUGR children.

The study of Wjst et al (9) evaluated the effects of both intrauterine growth and prematurity on airway symptoms and lung functions in 2470 non-selected school-aged children. IUGR was not clearly defined, but there were at least 55 term IUGR children; 187 children were born prematurely.
Table 9. The thus far published studies on the effects of IUGR on lung function, bronchial responsiveness, and pulmonary morbidity. Only the studies in which the definition of IUGR has been based on the gestational age adjusted birth weights were included.

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Number of children</th>
<th>Perinatal data</th>
<th>Volumes</th>
<th>Expiratory flows</th>
<th>BHR present</th>
<th>Asthma incidence</th>
<th>Airway symptoms</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rona (16)</td>
<td>5573 (5-11)</td>
<td>Parental qst</td>
<td>Yes</td>
<td>Yes</td>
<td>ND</td>
<td>No</td>
<td>No</td>
<td>IUGR was linked with lowered lung function; prematurity was linked with respiratory symptoms</td>
</tr>
<tr>
<td>McLeod (47)</td>
<td>866 (8-9)</td>
<td>Recorded at birth, qst</td>
<td>No</td>
<td>No</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>VLBW, not IUGR, was linked with subnormal flows and lung volumes. VLBW was linked to increased respiratory morbidity and BHR</td>
</tr>
<tr>
<td>Wjst (9)</td>
<td>2470 (5-14)</td>
<td>Parental qst</td>
<td>No</td>
<td>No</td>
<td>Yes (Yes)</td>
<td>No</td>
<td>No</td>
<td>IUGR was a risk factor for BHR in children born at term</td>
</tr>
<tr>
<td>Present study (13-21)</td>
<td>67</td>
<td>Clinical record</td>
<td>No</td>
<td>Yes</td>
<td>(Yes)</td>
<td>No</td>
<td>No</td>
<td>IUGR was linked with subnormal flows and BHR, but not with lung volumes</td>
</tr>
</tbody>
</table>

ND not determined or not shown in the paper; qst questionnaire; BHR bronchial hyperresponsiveness; LBW low birth weight; VLBW very low birth weight (< 1500 g)
6.2. Intrauterine growth retardation and lung function

Rona et al (16) showed that IUGR was associated with decreased expiratory flows. In this respect, the present results are in same direction, though they were significant only for FEF50. In addition, the lung volumes were lower in IUGR children, which is in disagreement with the present results. The epidemiological data of Rona et al, although extensive including 5573 children, were solely based on parent recall of birth weights and gestational weeks. In the present cohort study, the questionnaire data were checked against the medical cards of the hospitals and health care centers, and the exact gestational weeks and birth weights were known. Since all subjects were born at ≤ 38 gestational weeks, our study design did not allow for any separate evaluation of the effects of prematurity.

In the study of McLeod et al (47), VLBW children had lower FEV% and lung volumes than controls. The proportion of IUGR was 34 % of all VLBW children. The authors proposed that IUGR was not connected to abnormal lung volumes or flows, but the results of the IUGR children were not reported separately. Birth weights and gestational ages were exactly known, but the case-control design and the distribution of the birth weights differed from the present cohort study.

In the study of Wist et al (9), 2470 non-selected school-aged children were divided into four categories: 1) preterm (birth < 38 gestational weeks) and low birth weight (≤ 2500 g) children, 2) preterm and normal birth weight (> 2500 g) children, 3) term and low birth weight children and 4) term and normal birth weight children. Birth weights were exactly known, but the gestational ages were classified as < 38 weeks or more. The lung
volumes were reduced and the expiratory flows were normal in preterm LBW children. The flows tended to be reduced in full term LBW children. In the present study, volumes were within the normal range in preterm children irrespective of the intrauterine growth pattern, but one flow parameter were lowered in IUGR children. However, the comparison of these two studies is problematic due to the differences in the classification of the cases and in the study designs.

The results of Wjst et al and Rona et al are in agreement with the Barker’s hypothesis, whereas the results of McLeod et al and the present results are mainly in disagreement. Similarly in other studies, the results have varied. Low birth weight has often been associated with subnormal lung function in many studies (4, 14, 15, 46, 47, 75, 128, 129), but not in all (3, 130). The lung function subnormalities have usually consisted of lowered expiratory flows (4, 14, 15, 46, 75, 128), and in the minority of studies of lowered lung volumes (46, 47, 129). These studies have considered only LBW infants, not reporting separately the effects of prematurity and intrauterine growth retardation.

6.3. Intrauterine growth retardation and bronchial hyperresponsiveness

In the present thesis, preliminary evidence was found that IUGR may be associated with later BHR. In 1993, 60% of the MIC-responders, and 70% of those who responded in two or three challenges were earlier IUGR children. All the cold responders in the 1999 follow-up study belonged to the IUGR group. Overall, no differences were found in reactivities to methacholine, cold air and exercise by free running attributable to intrauterine growth pattern in this study.
BHR declined with age, but IUGR children did not manage as well as AGA children. In the AGA children, the cold responsiveness decreased from 21% to 0% during the 6-year follow-up through puberty. No significant decrease was to be seen in the IUGR children. Thus, our results offer preliminary evidence that intrauterine growth retardation may be a risk factor for permanent BHR, but the numbers of reactive subjects were too small to allow one to make any firm conclusions.

As discussed earlier, there are only three studies in which birth weights have been either adjusted for gestational age (9, 16) or the effects of gestational age have been taken into account in some other way (47). In addition, the BHR results have been reported in only two of these studies (9, 47).

In the study of McLeod et al (47), BHR was evaluated by a free indoor running test. The authors did not report the results in IUGR children separately, but the children with birth weight < 1500 g were significantly more likely to experience BHR. In addition, these at birth VLBW children used later more inhaled drugs for asthma or wheezing. In the present study, the four IUGR subjects who continued to have BHR through puberty, had weighed from 1050 g to 2120 g when newborns, and two of them still had wheezing symptoms and possible asthma.

Wjst et al. (9) found an increased cold air reactivity in children with low birth weight, but only if they were born at term. In contrast, in preterm children, the birth weight 2500 g was not a threshold for increased bronchial responsiveness. They found that BHR was associated more with low birth weight than with prematurity. Thus, mild prematurity alone may not be a risk for pulmonary impairment. The term LBW
children in the study of Wjst’s et al can be regarded as equivalent to IUGR children; the 2500 g weight in term newborns represents the -2 SD level in the present study. The results of the present study, on average, are opposite to Wjst’s results. However, earlier IUGR children were over-represented in both the MIC and IHCA responders, as well as among those who responded to multiple challenges in the present study.

In other studies, low birth weight has been linked to BHR (15, 72, 131), but again not in all studies (75, 128-130). The problem in all of these studies has been that low birth weight may reflect either prematurity or intrauterine growth retardation. A large study evaluating over 20,000 army recruits showed that low birth weight was linked to asthma (1). The result was similar in a study among 5600 < 10 years old children (11). BHR was not experimentally measured in either of these studies.

At the onset of the present study, none of the subjects had asthma. However, the medical histories and responses in the cold air challenge test suggested that two subjects probably did have asthma, and they were earlier IUGR twins. In the study of McLeod et al (47), VLBW was associated with wheezing illnesses. In the study of Rona et al (16), IUGR was not connected with asthma or wheezing. In addition, Wjst et al (9) found that asthma prevalence was not associated with low birth weight in term children. They found that hyperreactive airways, not expiratory flows or lung volumes, were more clearly associated with low birth weight rather than with prematurity.
6.4. General methodological discussion

The children, recruited from multiple births from a well defined area during an eight-year collection period, represent only 65% of all of the eligible IUGR/AGA twin pairs. On the other hand, this is a group of children in whom the effects of intrauterine growth retardation, if present, are expected to be most easily confirmed. Furthermore, the children had been treated in the same neonatal intensive care unit with the same treatment principles.

The prevalence of bronchial cold responsiveness in the first survey was quite high, 29%, but within the range of other BHR studies (65). In the follow-up study, the prevalence of cold responsiveness was lower, again in accordance with earlier findings, in which a decreasing trend of BHR has been found between 9 to 15 years of age (86). As expected, the cold air challenge and free outdoor exercise tests mainly identified the same children, as these tests probably are built on a common pathophysiological basis. An association between BHR and postneonatal infection parameters was found in the present study, as has generally been found in other studies (85, 132).

At the start of this work, none of the subjects in the study group had been diagnosed as having asthma. Thus, it was not possible to evaluate any associations of perinatal and neonatal factors with asthma and asthma suggestive symptoms. Therefore, the conclusions concerning pulmonary morbidity are based on only lung function results and bronchial reactivity.
Preliminary evidence was found that IUGR may be associated with later BHR. These findings are to some extent in accordance with Barker’s hypothesis of “prenatal programming of pulmonary health”. However, the main result was against Barker’s hypothesis; IUGR was not associated with decreased lung volumes or expiratory flows. It is worth noting that the duration of the follow-up of the present study, though it did continue until early adulthood, may be too short to conclusively evaluate Barker’s hypothesis. To be optimal, the follow-up should continue until mid to late adulthood to identify all aspects, including proness to chronic obstructive pulmonary disease, of possibly IUGR-related pulmonary morbidity.
7. SUMMARY AND CONCLUSIONS

1. The lung functions were similar in the IUGR and AGA children, when studied at school age. Only FEF50 values were lower in the IUGR than in the AGA children. All other expiratory flows and the lung volumes did not differ on the basis of the intrauterine growth pattern. Thus, routine follow-up of lung function is not essential in IUGR children.

2. Preliminary evidence was found that IUGR may be associated with later BHR. On average, no differences were found in reactivities to methacholine, cold air and free running between IUGR and AGA children. However, 60 % of the MIC-responders and 70 % of the responders in two or three challenges were former IUGR children. In addition, all cold responders in the follow-up study were IUGR children at birth. More studies are needed to prove or disprove the link between IUGR and BHR.

3. The reactivity to cold diminished during adolescence, but only in AGA children. Thus, BHR, when present in IUGR children, may be permanent, continuing into adulthood.

4. The cold air challenge test and free running test outdoors identified mainly the same responding children. The free running test outdoor is recommended as being a primary approach for children in whom bronchial challenge test is indicated.
8. REFERENCES


