

# Lung function and bronchial responsiveness in young children

Clinical and research applications

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## 1. INTRODUCTION

Longitudinal cohort studies suggest that outcomes such as lung function and bronchial hyperresponsiveness (BHR) in schoolchildren and adults with asthma probably are determined primarily in early childhood (8-11) and possibly already in infancy (12). Moreover, judgment of remission based solely on symptoms may overlook sub-clinically active disease (13). Likewise in cystic fibrosis (CF), significant reduction in lung function have been found at a very early age (14-18), even in the absence of clinically recognized lung disease (19). Confronted with these prerequisites the reasons for early and longitudinal measurement of lung function and BHR are as obvious as the goals: to enable early diagnosis and intervention, and improve prognosis in asthma and CF.

There are some lung function tests available for infants, while methods for measurements from infancy until age six are sparsely reported. The techniques used in infancy are not applicable and spirometry rarely produces reliable results in preschool children (20). This led, from the early nineties, researchers to perform thorough evaluations of several techniques by which lung function measurements are carried out during normal tidal breathing in awake young children not requiring reproducible forced maneuvers, but only the passive co-operation of the child, independent of the child's ability to follow verbal instructions. These investigations established and described applicability, repeatability, sensitivity, normal reference values, advantages and limitations in details, but did not focus on the clinical usefulness of primarily three individual lung function methods: whole-body plethysmography measuring specific resistance of the airways (sRaw), interrupter technique measuring resistance of the respiratory system (Rint) and impulse oscillation technique (IOS) measuring resistance (R) and reactance (X) of the respiratory system at 5 Hz ( $R_{rs5}$  and  $X_{rs5}$ ) (21-28). These methods all differ in their technical approach, but basically they assess respiratory mechanics by measuring the relationship between respiratory airflow and the pressure generating this airflow.

Bronchial hyperresponsiveness (BHR), defined as an abnormal increase in airflow limitation following a relevant stimulus to the airways, is a major pathophysiological phenomenon of bronchial asthma (29-31). However, the exact relationship between BHR and asthma on one side (30) and the relevance of performing bronchial provocation testing as part of the diagnostic work-up (32) or management (31) on the other, has not been completely outlined. Indeed BHR may only disclose presence of underlying airway inflammation, and not the exact nature of the disease since BHR is also

associated with other lung conditions such as CF and bronchopulmonary dysplasia in children born prematurely and with viral respiratory infections and allergic rhinitis (33).

Nonetheless, there is a definite association between asthma and BHR (29), which has been known for ages, studies on this phenomenon in young children has been complicated for the same reasons given for the lack of reasonable methods of lung function testing. However, methacholine challenges (MCh) were introduced in combination with forced oscillation technique (FOT) back in 1986 with some success (34), but over the years different techniques, either alone or in combination, ranging from indirect lung function tests such as transcutaneous oxygen tension ( $P_{tcO_2}$ ) (22, 24, 35, 36), auscultation (37, 38) to direct tests such as Rint (22, 24, 39-41) and sRaw (22, 24, 42) have been evaluated. However, direct bronchial challenge techniques such as MCh are probably not mimicking the pathophysiology of asthma to the same extent as indirect tests (30), which soon attracted our attention in looking to be able to perform simpler and less time consuming indirect bronchial challenges in young children enabled by IOS, Rint and sRaw as response measures. Interestingly, indirect tests are generally old and well investigated in school children and adults, but have once again achieved revival and have during the later years been offered intensified and major attention from investigators within the field of asthma, primarily due to the closer relationship with asthma pathophysiology (29-31). In fact, in 1998 the European Respiratory Society (ERS) approved a Task Force on Indirect Airway Challenges. Recommendations from this work was recently published (30). Furthermore, demonstration of BHR may not only serve as a specific diagnostic feature of asthma, but also function as a special feature in phenotyping infants (12, 43) and preschool children (44) with asthmatic symptoms, and in this respect serve to specifically achieve improvement in control of asthma from phenotype-specific treatment (45, 46). Disclosure of BHR may even carry a potential for prediction of persistency of asthma during preschool age (47) or from school age into adulthood (9).

Thus, the overall purpose of the research presented in this thesis was to evaluate the clinical application of sRaw, Rint and IOS in different settings and to further elaborate the possibility to develop a simple, indirect bronchial challenge test to measure bronchial responsiveness (BR) and demonstrate BHR in preschool children. This paper reviews the evidence on the clinical applications, reliability and feasibility of whole-body plethysmography (sRaw), interrupter technique (Rint) and impulse oscillometry (IOS) for measurements of lung function, and of hyperventilation techniques in assessment of BR in healthy children and in children with chronic lung disease, from two years of age.

## 2. AIMS

It was the principal aim of this thesis to evaluate the clinical application and discriminatory capacity of whole-body plethysmography, interrupter technique and impulse oscillometry as lung function tests in healthy children, asthmatics and children with cystic fibrosis of preschool age.

The primary aim was to develop and implement a test for indirect bronchial challenge and assess its validity as judgement of bronchial responsiveness.

The secondary aim was to compare tests for measurement of baseline lung function and bronchial responsiveness.

The tertiary aim was to validate such tests through the studies of documented efficacious anti-asthma medications in asthmatic young children.

## 3. SUBJECTS AND METHODS

### 3.1 SUBJECTS

#### *Number, age and recruitment*

376 patients and healthy subjects participated in the different studies (1-7). Principally all children were 2 to 5 years (1-5, 7) i.e.

young or preschool children, but in the CF study (6) the children were 2 to 7 years at inclusion. All children with CF and the majority of asthmatics were recruited from the outpatient clinic at the department of pediatrics, pulmonary service, Rigshospitalet, while some asthmatics came from a private pediatric asthma clinic. The parents of all patients and healthy controls gave written informed consent to the study protocols, which were approved by the local ethics committee of Copenhagen.

### 3.1.1 Patients

#### *Asthma*

Asthma was empirically diagnosed on the basis of recurrent asthma symptoms, clinical improvement with regular inhaled corticosteroid (ICS) therapy, and relapse during interruption of such treatment.

#### *Cystic fibrosis*

All patients had a positive sweat test and genotype diagnosis of CF.

### 3.1.2 Healthy controls

Normative lung function data have been previously collected (25) while additional normative data on lung function, and responsiveness to hyperventilation of cold air (1) and bronchodilators (5) were collected in a separate group of healthy controls. Children without any previous lung illness, atopic symptoms, atopy in first-degree relatives, or smoking at home qualified as healthy controls. The healthy controls were collected by a health questionnaire which was mailed to families of young children 2 to 5 years of age living in Greater Copenhagen. The families were randomly selected from the municipal population database.

## 3.2 METHODS I: LUNG FUNCTION

### 3.2.1 Basics of the techniques

#### 3.2.1.1 Whole-body plethysmography

Whole-body plethysmography, introduced by DuBois in 1956 (48, 49), was used for measurement of specific airway resistance,  $sRaw$  (1-7). Airway resistance ( $Raw$ ) is expressed by a formula resembling Ohm's law – alveolar pressure divided by airflow. Alveolar pressure is reflected by minute changes in plethysmographic volume (using Boyle's Law) during inspiration and expiration and airflow is measured with a pneumotachograph. In DuBois' original method  $Raw$  is calculated from dividing  $sRaw$  by the thoracic gas volume (TGV). First,  $sRaw$  was measured from simultaneous recording of airflow and plethysmographic volume changes during tidal breathing and second, TGV was determined from recording of plethysmographic volume changes and the corresponding mouth pressure changes during tidal inspiratory and expiratory breathing against a closed shutter. The latter is not tolerated by young children. Dab et al (50, 51) and later others (52) took advantage of a simple algebraic manipulation of the formulas which precluded the separate measurements of  $Raw$  and TGV omitting this latter step and thereby provided measurements of  $sRaw$ , which greatly facilitates the procedure even in young children.

$sRaw$  reflects the overall dimensions of the airways, including the effect of lung expansion on the caliber of the airways. Since  $sRaw$  is the product of  $Raw$  and TGV, it cannot distinguish if improvements or deteriorations are due to either of its two components. It follows, that any increase of  $Raw$  or increase of TGV or both, results in a high, abnormal  $sRaw$  value. Similarly  $sRaw$  will decrease if either  $Raw$  or TGV or both are diminished and finally, a normal  $sRaw$  indicates that both  $Raw$  and TGV must be in a normal range (51).  $sRaw$  is more dynamic than  $Raw$  as shown by comparing  $sRaw$  and  $Raw$  in asthmatics and healthy children (53).

Plethysmographic measurements of  $sRaw$  have been successfully adapted and approved for use in children from 2 years of age in a still increasing number of studies in asthma (1-5, 21-27, 47, 54-56) and in CF (6, 57). The use of an adapted face-mask, and an adult accompanying the child in the box has increased acceptance of this method

(23). We recently published a comprehensive review of plethysmographic measurements in children from 2 years of age (58).

#### 3.2.1.2 Interrupter technique

Interrupter technique was used for measurement of respiratory resistance,  $R_{int}$  (1, 3, 5, 6). Modern computers have allowed a reappraisal of this technique and a better understanding of the physiology underlying the measurements (59, 60) resulting from this old technique, first described by Neergard and Wirz in 1927 (61). The interrupter technique entails rapid and complete occlusion of the airways (by closing a valve or shutter) during a normal breathing cycle while flow and airway opening pressure (mouth pressure =  $P_m$ ) are recorded (59, 60, 62). The method is based on the assumption that alveolar pressure ( $P_A$ ) and  $P_m$  equilibrate instantaneously at airway occlusion (behind the occlusion). Dividing this pressure change by the flow, recalling Ohm's law, allows the simple calculation of  $R_{int}$ ,  $R_{int} = P_m/\text{flow}$ . The initial rapid pressure change is followed by a second slower change in pressure which is related to the visco-elastic properties of the respiratory tissues, together with any gas redistribution that occurs between lung units following the occlusion (62). Several studies have employed  $R_{int}$  measurements in healthy children, wheezy and/or asthmatic young children and young children with CF looking to test baseline lung function, responsiveness to bronchodilators and bronchial challenge tests (1, 3, 5, 6, 21, 22, 24-26, 63-65).

#### 3.2.1.3 Impulse oscillometry

Impulse oscillometry was applied to measure  $Xrs_5$  and  $Rrs_5$  (1, 3, 5, 6). IOS, often referred to as a measurement of respiratory input impedance ( $Zrs$ ) is, like  $sRaw$  and  $R_{int}$ , a non-invasive method feasible for measurement of airway function in young children (28, 66). However, a characteristic feature of IOS, in contrast to both  $sRaw$  and  $R_{int}$ , is that measurements are not based on respiratory flow and pressure signals generated by the subject, but on signals from the pressure-flow relationship (output) of artificial impulse-shaped test signals (input) which are produced by an external generator (loudspeaker) and superimposed on the patient's respiratory system and respiratory tidal breathing waveform (67). The transducers for flow and mouth pressure which are connected to a pneumotachograph register the respective signals of total pressure and total flow consisting of the portion of spontaneous breathing and the superimposed impulse signal. In order to determine  $Zrs$  the impulse signals have to be separated from the breathing by means of signal filtering. This is done via time and frequency analysis by Fast Fourier Transformation. IOS is a variant of FOT introduced by Dubois et al (68) to characterize  $Zrs$ , defined as the instantaneous ratio between pressure ( $P$ ) and airflow ( $V'$ ), over a wide range of frequencies.  $Zrs$  can be divided into respiratory resistance ( $Rrs$ ) and reactance ( $Xrs$ ) depending on whether the peak of pressure and peak of flow is in or out of phase, respectively. The values of  $Rrs$  and  $Xrs$  are dependent of frequency of the applied oscillations. IOS yields values similar, but not identical to those provided by FOT (69). The method is easy to use in young children (1, 3, 5, 6, 21, 22, 24-27, 70) and attractive to many researchers, especially in the USA (71-73) but publications on measurements in young children are still limited.

#### 3.2.1.4 Transcutaneous $O_2$ and $CO_2$ Tension

Transcutaneous  $CO_2$  tension ( $PtcCO_2$ ) and  $CO_2$  tension ( $PtcCO_2$ ) was applied during bronchial challenge testing (1, 3). Blood gases are released to the skin through the capillaries. The electrode heats up the skin.  $O_2$  and  $CO_2$  diffuse through the skin and the membrane to the cathode where a reduction occurs and a current is generated. The signal is then sent to the microcomputer and converted into  $PtcO_2$  and  $PtcCO_2$  readings.

#### 3.2.1.5 Spirometry

Spirometry was used for measurement of  $FEV_1$  and FVC. Modern

spirometry employs pneumotachographs which in principle detects flow from the pressure difference over a small, fixed resistance (R) offered by a fine metal screen or mesh as e.g. the Lilly screen type pneumotachograph. The pressure difference across the mesh ( $P_1-P_2$ ) relates linearly to flow ( $V'$ ) at relatively low flows, when the flow pattern is laminar. Higher flows give rise to a turbulent flow pattern but the special designed head of the pneumotachograph has a trumpet-like configuration assuring laminar flow over a wide range of flows. Flow ( $V'$ ) is then derived from the formula:  $P_1-P_2 = R \cdot V'$ .

### 3.2.2 Equipment, nose-clip, face-mask and mouthpiece

#### Equipment

Pulmonary function testing was performed with a Master Screen unit, version 4.34 (E. Jaeger GmbH, Würzburg, Germany). This unit integrated all the lung function techniques, sRaw, Rint, IOS and spirometry, in one program. Flow and volume were measured with a heated pressure screen-type pneumotachograph with a resistance of  $0.036 \text{ kPa} \cdot \text{s} \cdot \text{L}^{-1}$  (sRaw, Rint, IOS and spirometry). The equipment was calibrated daily.

sRaw measurements employ a constant volume (830 L) whole-body plethysmograph (body-box), which in principle is a sealed cabin built for use in adults (Figure 3.2.2). The cabin is equipped with a pressure transducer that records pressure changes.

Rint measurements require a shutter-device (E. Jaeger GmbH, Würzburg, Germany) mounted on the pneumotachograph (Figure 3.2.2.). Commercially available equipment allows the measurement of Rint at set flows or set volume during either inspiration or expiration. The shutter is automatically and rapidly activated and able to instantaneously and completely disrupt the flow through the airways and the pneumotachograph for a preset period, often 80 to 100 ms before allowing normal respiration to resume. Pm was measured by a transducer placed in the shutter.

IOS requires an Impulse Oscillometry System (E. Jaeger GmbH, Würzburg, Germany) consisting of a pneumotachograph supplied with two differential pressure transducers connected through a Y-adaptor to an impulse generator (loudspeaker) and a terminating resistance, respectively (Figure 3.2.2).

PtcO<sub>2</sub> and PtcCO<sub>2</sub> were measured by a blood gas monitor, Radio-

meter TCM 3 (Radiometer, Copenhagen, Denmark). Calibration was performed with a calibrating gas containing 20.9% O<sub>2</sub> and 5% CO<sub>2</sub>. The temperature of the electrode was 44°C during measurements. All measurements were corrected to 37°C.

Further details on various equipments are given elsewhere (21, 22).

#### Mouth piece/face mask

Spirometry requires the subject to wear a nose-clip and keeping the lips sealed around a mouth-piece. In young children such requirements detract from acceptance. With a standard face-mask the child is likely to breathe through the nose. We therefore used a special adapted face-mask with a large cushion and a built-in flexible tube, which prevented nasal breathing by assuring that the mouth remained open and assured a good seal and stabilization of the cheeks and chin (21). The latter is of importance for IOS and Rint measurements since presence of a compliant compartment between the resistive airways and the mouth may interfere with results. The major compliant pathway in children is the extra-thoracic airway and cheeks, and measurements are routinely made with supporting the subject's cheeks in an attempt to minimize these problems. Specifically Rint measurements may be buffered during the initial rapid "resistive" pressure change, making calculation less accurate. These effects are potentially more clinically important in patients with markedly increased airway resistance (74) or during bronchial stimulation where resistance is deliberately changed. Although repeatable Rint measurements can be obtained from young children using either a mouthpiece or a face-mask, there are significant clinical and statistical differences between the results obtained, preventing the two methods from being used interchangeably (75) Our specially adapted face-mask (21) fulfills elegantly the need for a standardized support of cheeks in contrast to cheek support by hands (child's or investigator's) used in other studies (76-78).

### 3.2.3 How to measure and practical aspects

#### Whole-body plethysmography

Flow ( $V'$ ) in the airways and variations of the pressure in the plethysmograph was simultaneously measured as the child was breathing through the pneumotachograph while seated inside the



**Figure 3.2.2.**  
Upper left: Whole-body plethysmography. Upper right: Interrupter technique. Lower left: Impulse Oscillometry System. Lower right: Cold air challenge system.

plethysmograph (Figure 3.2.2). Volume and pressure in the plethysmograph was calibrated routinely before measurements whereby changes in plethysmographic volume ( $\Delta V_{\text{pleth}}$ ) were measured through changes in box pressure. The simultaneous recordings of  $V'$  and  $\Delta V_{\text{pleth}}$  were depicted as the specific resistance loop and the inserted straight parameter line from which  $sRaw$  was calculated, Figure 4.1.2.a.:  $sRaw = (\Delta V_{\text{pleth}}/\Delta V') \cdot (P_{\text{amb}} - P_{H_2O})$  where  $(\Delta V_{\text{pleth}}/\Delta V')$  corresponds to  $1/\tan \beta$ , where  $\beta$  is the closed angle between the x-axis and the line (parameter line) drawn through the s-shaped resistance loop (see also 3.2.4),  $P_{\text{amb}}$  is ambient pressure,  $P_{H_2O}$  is the pressure of water vapor at body temperature, and  $P_{\text{amb}} - P_{H_2O}$  is the approximate thoracic gas pressure.

To ensure slight neck extension and thereby preventing airway compression from the neck as well as to improve the co-operation, a monitor showing video cartoons was placed in front and above the subject during all measurements (21). Communication via microphone and loudspeaker ensured instruction of the child and adult during testing. Since  $sRaw$  measurements were performed with automatic electronic BTPS compensation shown to exhibit positive frequency dependency (23, 42, 79) all measurements were made at recommended breathing frequencies from 30 to 45 (23) as a standard procedure to eliminate influence of breathing frequency in the measurements before and after different stimuli (1-7). If the child refused to enter the plethysmograph alone or otherwise was uncooperative, measurements were attempted with an adult person accompanying the child inside the plethysmograph (23, 80). Such measurements exhibit good correlation with values obtained from the child alone (23, 80) The adult performed a constant slow expiratory maneuver for a period of 20 s allowing sufficient time for measurement of  $sRaw$  of the child. Such maneuver gives rise to a slight continuous drift of the signal measuring changes of plethysmographic volume, which is corrected for by the data processing software together with the drift from temperature build-up and easily separated from the more rapid pressure changes from the child's breathing.

#### *Interrupter technique*

$R_{\text{int}}$  (1, 3, 5, 6) measurements were performed as previously described (21). On every second inspiratory phase, inspiration of 50 mL activated and closed the shutter for 80 ms. Mouth pressure was measured during the last 5 ms of the interruption. Flow was measured over 5 ms, starting 70 ms after reopening (28). During recent years there has been extensive debate on whether inspiratory or expiratory  $R_{\text{int}}$  should become the standard method. Theoretically there are expected physiological variations between inspiration and expiration, with airway resistance being lower during inspiration due to the airway being "pulled open" by the forces of interdependence, supported from studies in school-age (74, 81) and young children (82). However, in practice, neither flow nor volume dependence of resistance have been found to be important effects during the tidal volume range in young children (83, 84) or school children (85). Most investigators seem to be adopting the practice of measuring  $R_{\text{int}}$  during the expiratory phase of respiration at peak tidal flow (86, 87). However, this choice seems to be more for practical reasons than based on evidence. The child was seated upright, if needed on the lap of the parent, who could also be instructed to perform the application of the equipment. Slight neck extension was ensured in the same way as described for  $sRaw$  measurements. The child should breathe calmly and regularly. Fast or irregular breathing was not accepted, but in contrast to  $sRaw$  and IOS measurements no frequency dependence has been described for this method.

#### *Impulse oscillometry*

Measurements in our studies (1, 3, 5, 6) were performed throughout the respiratory cycle and as previously described (21, 22) and later adopted by other groups (72, 73, 88, 89) At least 30 s of undisturbed

breathing resulting in technically satisfactory tracings of pressure and flow measurements were used as outcome. The child was seated as described for  $sRaw$  and  $R_{\text{int}}$  and asked to breathe calmly and regularly aiming for a rate of 20 to 40 breaths per min.  $R_{\text{rs}}$  and  $X_{\text{rs}}$  were calculated in the frequency range from 5 to 35 Hz of each pressure pulse (0 to 100 Hz) at every 0.2 s.

#### *PtcO<sub>2</sub> and PtcCO<sub>2</sub>*

After calibration the electrode was placed on the flexor side of the left forearm. Typically, steady-state readings on the printed hard-copy curves were seen after approximately 20 min and measurements then began after 5 min.

#### *Spirometry*

Measurements were performed with the patient in sitting position wearing a nose-clip and using a mouthpiece, while holding head straight and in slight extension. First the child was breathing spontaneously, while adapting to mouth-piece and nose-clip. On instruction the child performed a maximal deep and forced inspiration and without pause the child then expired as rapidly and deeply as possible, while verbally encouraged by the assistant.

### **3.2.4 Quality control of measurements**

#### *Whole-body plethysmography*

Measurements awaited temperature stabilization in the box (~1 min) and specific resistance loops were not collected before regular tidal-volume curves were seen. On-line display of the loops allows detection of artifacts such as abnormal patterns caused by swallowing, vocalization, breath-hold, coughing or leakage around the face-mask (23). The decision whether to accept or reject a measurement was taken during measurements.  $sRaw$  is calculated as the median value of 5 consecutive and technically satisfactory loops. Different estimates of  $sRaw$  may be calculated from the loop depending on the protocol for the application of the parameter line on the loops (58). We used  $sRaw_{\text{tot}}$  where the parameter line connects the flow points at maximum change in plethysmographic volume (pressure), Figure 4.1.2.a (1-7).  $sRaw_{\text{tot}}$  seems to be at least as sensitive as other methods in estimating the total resistance, though this estimate may have a higher variability (27).

#### *Interrupter technique*

The continuous tidal volume curve was shown on screen as measurements proceeded and after each interruption the pressure versus time and flow versus time curves were displayed. The procedure for validating  $R_{\text{int}}$  measurements was the same in all studies (1, 3, 5, 6). First, all  $R_{\text{int}}$  values from breathing cycles disturbed by a respiratory pause, swallowing, speaking, moving, or coughing; or accompanied with a flagrant  $P_{\text{m}}$  curve error (blunted initial rapid changing phase, lack of oscillations, or aberrant second changing phase) were discarded immediately. Second, the time traces of  $P_{\text{m}}$  and flow were examined and only when 5 consecutive equal appearing traces were seen, the measurement was accepted, and *mean*  $R_{\text{int}}$  automatically calculated and saved as the result (21, 22). A recent paper has shown that mean and median expiratory  $R_{\text{int}}$  values are not significantly different (82). However, since the  $R_{\text{int}}$  values obtained during a measurement session are not normally distributed, it has later been suggested that *median* values should be used as they are theoretically more correct (77).

Our  $R_{\text{int}}$  estimate is described in (3.2.3). Different estimates of  $R_{\text{int}}$  can be calculated from the  $P_{\text{m}}$ - and flow-curves depending on which parts of the curves that are chosen for calculation (40, 90). It is now common to pre-program the interrupter device to interrupt the airflow during tidal expiration at either peak flow or at a predetermined flow e.g.  $0.2 \text{ L}\cdot\text{s}^{-1}$  corresponding to maximal tidal expiratory flow in young children. The estimated  $P_{\text{m}}$  is then found by linear back-extrapolation of the post-occlusion signal (typically at 70 and 30 ms after closure) to e.g. 15 ms after closure (40, 41, 63, 74,

77, 81, 83, 91-93). Rint is calculated by dividing Pm by the pre-programmed flow at occlusion. The Rint value is significantly dependent on the algorithm used (90).

#### *Impulse oscillometry*

Computer software for real-time analysis of flow and impedance versus time were used during data acquisition in order to evaluate signal quality. Data validation was the same in all studies (1, 3, 5, 6) and was based on a visual examination of the pressure and flow tracings, and if accepted, a following automatic calculation performed by the computer gave a list of Rrs and Xrs values at different frequencies. If the tidal breathing curve was disturbed by a respiratory pause, swallowing, speaking, moving, or coughing the tracing was continued until 30 s of undisturbed tracing was seen. The automatically calculated values for Xrs<sub>5</sub> and Rrs<sub>5</sub> were used as the result. However, re-analysis of the tracings is possible for other frequencies if needed (21, 22).

#### *PtcO<sub>2</sub> and PtcCO<sub>2</sub>*

The mean value of four readings at 1-min intervals on the hard-copy curves printed during measurements of PtcCO<sub>2</sub> were used as the result (1). The data used for calculation of PtcO<sub>2</sub> (3) after each step of the MCh were 5 point-readings on the curve at 1-minute intervals over a 5-min period immediately after completing the inhalation. The median value was used as the result.

#### *Spirometry*

In at least two trials the expiratory part of the flow-volume loop should be identical. Measurements were as per American Thoracic Society (ATS) standards; that is, the difference between the two highest values of FVC and FEV<sub>1</sub> should ideally be less than 200 mL and forced expiratory time at least 6 s (94).

### **3.2.5 Reliability**

#### *Whole-body plethysmography*

Short term repeatability within-observer is slightly dependent on the chosen estimate of sRaw. The total standard deviation (SD<sub>tot</sub>) for sRaw is in the range from 0.20 to 0.21 kPa·s with its constituents the within-subject SD (SDw) and between-subject SD (SDb) ranging from 0.086 to 0.109 kPa·s and 0.19 to 0.20 kPa·s, respectively. The within-subject coefficient of variation (CVw%) is in the range of 8-11% (23, 25, 50). Repeatability is independent of age (25) and measurements of sRaw with and without an accompanying adult do not alter repeatability (23).

Long term data on repeatability in healthy young children do not exist. Data on long term repeatability of sRaw 1 and 2 months apart in young asthmatics (3), are available but have not been analyzed. However, repeatability between occasions 1 month apart in young children with CF showed an ICC of 0.65 (6), which is considerably lower than the short term variability between measurements in healthy children (25), but comparable with long term findings on repeatability of e.g. Rint in children with persistent cough or previous wheeze (86). This suggests that long term repeatability in symptomatic children might be more influenced from the variability of the disease than the variability of the technique.

SDw of measurements obtained by two trained observers is greater than the within-observer variability. This between-observer variability of sRaw appeared to be reduced when sRaw was calculated as sRaw<sub>0.2</sub> or sRaw<sub>50%</sub> instead of the sRaw<sub>tot</sub> (27). Accuracy of sRaw has been evaluated against measurements under BTPS conditions, which showed that the available electronic BTPS compensation causes significant overestimation in children (23, 79).

#### *Interrupter technique*

Short term repeatability (minutes) in preschool children is acceptable (74, 76, 83) with CV% of 9% for controls and 7% for asthmatics (74) as compared to 8% in healthy (25) and 6.5% in a mixed popu-

lation of asthmatic and healthy young children (86). Repeatability is age dependent, with younger children having higher variability between measurements (76, 95). Beelen (87) reported a SDw of 0.10 kPa·s·L<sup>-1</sup> for short term variability under field conditions comparable to 0.08 kPa·s·L<sup>-1</sup> found by Klug (25). Within subject reproducibility of expiratory and inspiratory Rint expressed as intra-class correlation coefficients (ICC) was satisfactory with ICC values of 0.82 and 0.79 (77) and as high as 0.92 with our procedure (25).

Klug et al (25) did not report any long term repeatability. Long term (2.5 month) repeatability was 0.208 kPa·s·L<sup>-1</sup> for expiratory Rint when reported as 2 SDs of the mean paired difference between measurements (83). Beelen (87) reported a slightly higher SDw of 0.14 kPa·s·L<sup>-1</sup> under field conditions, but an identical SDw of 0.10 kPa·s·L<sup>-1</sup> under standard conditions as compared to short term data. Long term repeatability in symptomatic children gave no reason for optimism, since data from Chan (86) indicated that limits of agreement were too wide for change in the individual to be judged with confidence in contrast to the between occasion repeatability of FEV<sub>1</sub>. This might however be due to the variability of the disease rather than the variability of the technique as mentioned previously.

The random variability between observers appeared to be particularly high with the Rint method (SDw between observers was 0.212 kPa·s·L<sup>-1</sup>) in a comparison between sRaw, IOS and Rint (27), which was a major reason for not changing observer during any of the studies (1-7). However other investigators have published more impressive data on inter-observer agreement such as ICC values of 0.98 (77) and inter-rater reliability of 0.15 kPa·s·L<sup>-1</sup> (76).

Early studies found Rint to overestimate resistance in comparison to plethysmographic airway resistance (Raw) in healthy human adults (62). Curvilinear back extrapolation of Pm to the time of occlusion has been shown to improve Rint estimates derived from the Pm curve and the correlation between Rint and Raw in healthy adults (59, 96), while calculations with other algorithms gave significant overestimation. Comparisons of Rint with Raw in 20 school-children with mild to severe chronic airway obstruction was satisfactory in patients with FEV<sub>1</sub> > 60% predicted (77). Data on accuracy of Rint in young children are lacking.

#### *Impulse oscillometry*

Short term repeatability of Rrs<sub>5</sub> and Xrs<sub>5</sub> exhibited ICC of 0.85 and 0.79, respectively, and therefore acceptable (25). CVw% and SDw for Rrs<sub>5</sub> was 0.13 kPa·s·L<sup>-1</sup> and 10.2% and for Xrs<sub>5</sub> the SDw was 0.10 kPa·s·L<sup>-1</sup>, while calculation of CVw% was irrelevant due to values close to zero (25). Data on long term repeatability in healthy and asthmatic children are lacking. The inter-observer variability of Xrs<sub>5</sub> was similar to the within-observer variability (27). In contrast the variability of Rrs<sub>5</sub> between observers was high. Since both values are the result of one IOS tracing it is difficult to explain this discrepancy in variability by observer bias alone. Data on accuracy are very limited for this method. A study compared IOS with Raw in 49 subjects with a variety of lung disorders and found Rrs<sub>5</sub> to only moderately correlate with Raw and to markedly underestimate high resistance values (69).

#### *PtcO<sub>2</sub> and PtcCO<sub>2</sub>*

Measurements of PtcO<sub>2</sub> & PtcCO<sub>2</sub> are highly repeatable with CV% around 1.5% (39) to 2.3% (97) in asthmatic children.

#### *Spirometry*

CV% in school children is 5% and SD for values in percent of predicted is 10% (98).

### **3.2.6 Normative data**

#### *Whole-body plethysmography*

sRaw is independent of height and gender (25, 99-101).

Mean sRaw<sub>tot</sub> is 1.3 kPa·s (25).

### *Interrupter technique*

The revival of Rint for measurement of lung function in children has unfortunately been uncoordinated as reflected by an unrestricted number of publications on normative data and repeatability (25, 63, 74, 77, 78, 83, 84, 95, 102), showing that there is as yet no simple answer to what the most correct or reliable protocol is.

We used the first published normative data in young children (25) in our studies (1, 3, 5, 6), since securing identical Rint method and algorithm for group and longitudinal data is the only reasonable option, as recently recommended (90). Rint shows linear significant negative correlation with age, weight and height, but no gender difference (25, 63, 74, 77, 78, 83, 84, 95, 102) or difference between ethnicities (102).

Mean Rint is 1.04 and 0.9 kPa·s·L<sup>-1</sup> in children < and ≥5 years, respectively (25).

### *Impulse oscillometry*

Normative data for Xrs<sub>5</sub> and Rrs<sub>5</sub> was reported by Klug & Bisgaard (25). This reference material exhibited significantly higher Rrs and lower Xrs values as compared with two other pediatric reference samples (88, 89) using the same equipment and nearly identical measurement procedures except for the special face-mask, which probably explains the differences (28). Rrs<sub>5</sub> and Xrs<sub>5</sub> has been repeatedly shown to exhibit significantly negative and positive, respectively, correlation between age, height and weight (25, 88, 89, 103).

Mean values are, Xrs<sub>5</sub> = -0.44 kPa·s·L<sup>-1</sup> and Rrs<sub>5</sub> = 1.29 kPa·s·L<sup>-1</sup> (25).

### *PtcO<sub>2</sub> and PtcCO<sub>2</sub>*

Mean normal reference values for PtcO<sub>2</sub> and PtcCO<sub>2</sub> are 10.8 kPa and 5.1 kPa, respectively (104).

### *Spirometry*

We used reference values from Polgar and Promadhat (98), representing a robust dataset in good agreement with values from a recent study in preschool children, specifically in the interesting transition-zone from preschool to school age (105).

### **3.2.7 Acceptance**

More than 80% of healthy 2-7 year old children accepted all techniques, however with increasing success rate by age (25). Many children from 2 years of age can perform acceptable sRaw measurements. Particularly children familiar with the use of face-masks are successful from an early age. At the age of 2, 57% of untrained healthy children (N=28) completed the measurement (25). At the age of 3, 65% of untrained healthy children (N=31) completed measurements (25). Likewise, in a large birth cohort study of 766 children 66% completed measurements at age 3 (54). The main reason for failure to obtain measurements was that the child was unfamiliar with the face-mask (25). A short period of training can improve the acceptance. The success rate of Rint also increased by increasing age (25). Fifty-six per cent of 2 to 3 year-olds (n=79), 81% of 3 to 4 year-olds (n=104) and 95% of 4 to 5 year-olds (n=88) completed baseline testing, and 53%, 71% and 91%, respectively, completed reversibility testing with Rint (76). Feasible Rint measurements were obtained in 88% of 2-7 year old children (77). Success rate was 75% in preschool children (95) The success rate of IOS in both healthy (25, 89) and a mixed population of asthmatic and healthy young children was >83% (88). In contrast to the sudden occlusion of the Rint method, the sound of the loudspeaker in IOS was continuous and monotone, almost like the sound of an old train, and therefore more appealing to the young child. PtcO<sub>2</sub> & PtcCO<sub>2</sub> measurements did lead to minor local skin irritation from the electrode in 5 of 39 (13%) patients in one study (not reported) (3).

Acceptance of spirometry was not investigated since only accept-

able measurements were saved and only when the children were able to perform acceptable flow-volume loops. However we appreciate that some of the children might not have been able to meet the ATS criteria on forced expiratory time of ≥6 s, a common problem in childhood (106).

### **3.2.8 Safety**

No safety issues were raised or any complications seen with any of these measurements.

## 3.3 METHODS II: BRONCHIAL CHALLENGE

### *Introduction*

Bronchial challenges employ either direct or indirect stimuli to assess bronchial responsiveness (BR) and in particular bronchial hyperresponsiveness (BHR), a characteristic and major pathophysiological phenomenon of asthma (32). Direct stimuli such as inhalation of methacholine or histamine cause airflow limitation by direct action on airway smooth muscles. Indirect challenges probably induce airflow limitation by acting on inflammatory cells, epithelial cells and nerves, which upon stimulation release mediators or neurotransmitters that induce airway smooth muscle contraction (30). The first indirect test to be developed in adults and school children was exercise. Eucapnic voluntary hyperventilation was developed later, as a substitution for exercise. Hypertonic aerosols of saline or mannitol were introduced to mimic the dehydrating effects of evaporative water loss that occurs during hyperventilation (31).

### **3.3.1 Basics of the techniques**

#### *3.3.1.1 Cold Air Challenge (CACH)*

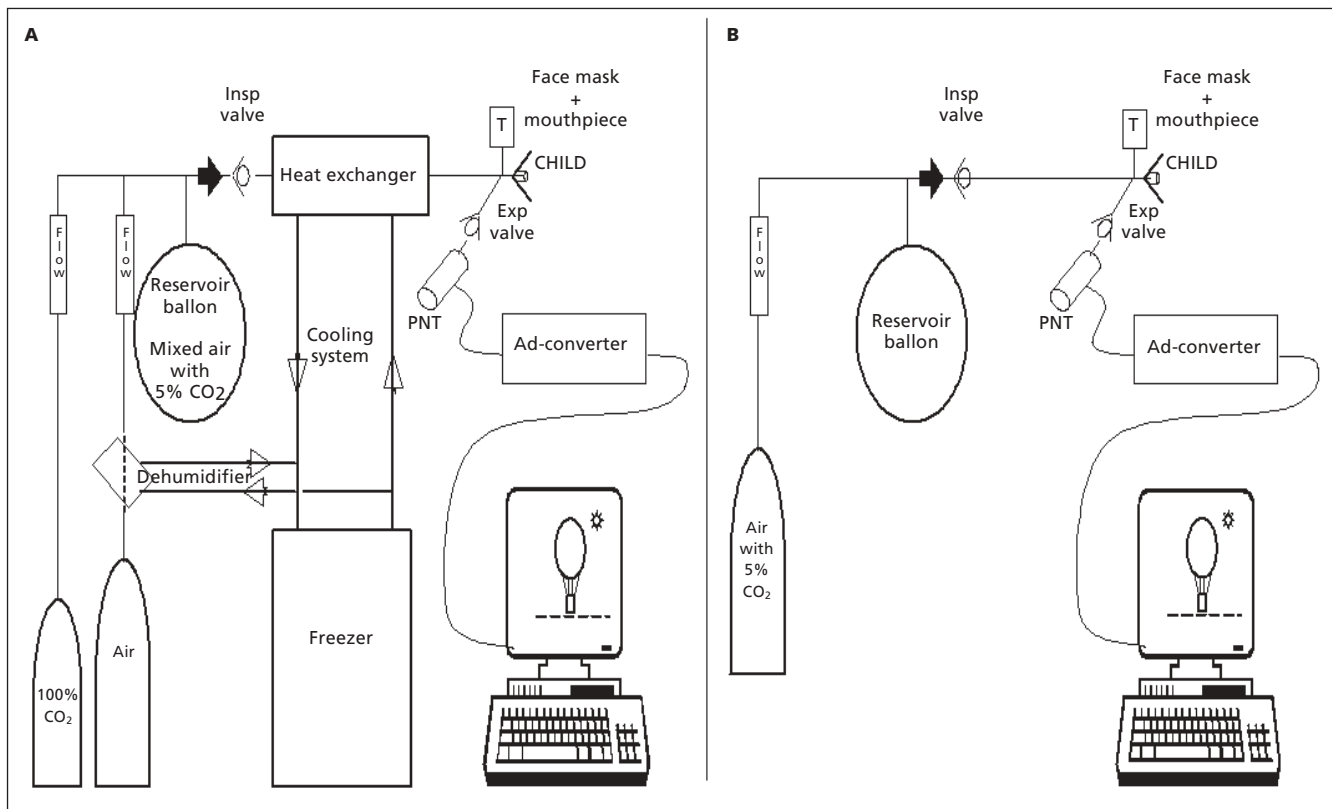
The major factors that determine severity of bronchoconstriction from eucapnic hyperventilation testing are the pulmonary ventilation reached and sustained during testing and the water content and temperature of the inspired air. The stimulus by which hyperventilation causes the airways to narrow is the loss of water in bringing large volumes of air to body conditions in a short time. The exact mechanism of airflow limitation is not fully understood, but probably involves heat flux and mucosal cooling from the hyperventilation (30) and/or transient hyperosmolarity with inflammatory and neuronal cells acting indirectly or directly, respectively, on bronchial smooth muscles (29-31). The relative importance of water evaporation and airway cooling is debated (29-31, 107-112). Kotaru and colleagues did cast some doubt on the premise for the osmolarity hypothesis by collecting airway surface fluid of normal subjects (113) and asthmatics (112) breathing dry, frigid air and then showed that hyperpnea had no influence on the amount of fluid recovered or its osmolarity.

#### *3.3.1.2 Dry Air Challenge (DACH)*

Bronchial challenge with dry air hyperventilation does not differ substantially from CACH in terms of basic mechanisms, although the component of deliberate intensified cooling of the airways is lacking, which may weaken the potency of the stimulus (108, 114).

#### *3.3.1.3 Methacholine Challenge (MCh)*

Delivering an aerosol to the airways with increasing accumulative dose or concentrations of agonists, such as methacholine or histamine cause airflow limitation via a direct effect on the effector cells involved in the airflow limitation, such as airway smooth muscle cells, bronchial vascular endothelial cells and mucus producing cells. Response is measured with any suitable lung function test, whether direct or indirect (see 4.1.2), and construction of the dose-response curve finally allows for definition of responsiveness. Different dosing protocols have been used. Each has advantages and disadvantages and the ATS committee on recommendations for MCh testing was unable to come to a single recommendation but narrowed the choices to two: (1) the 2-min tidal breathing method and (2) the five-breath dosimeter method (115).



**Figure 3.3.2.a.** Schematic presentation of CACH system & **b.** DACH system. See text for explanation.

### 3.3.2 Equipment, face-mask and mouth-piece

#### Cold Air Challenge (CACH) (1)

Generation of cold air was accomplished by a Respiratory Heat Exchange System (RHES) (E Jaeger GmbH, Würzburg, Germany), Figure 3.3.2.a and Figure 3.2.2. It is a mobile unit weighing 131 kg and dimensions: 55 × 75 × 175 cm. The RHES consists of a thermostatically controlled refrigerator operating in a temperature range +25°C to -25°C, using 5 L of ordinary alcohol mixed with water as coolant. The heat exchanger has low ventilatory resistance and the gas coming through and leaving the heat exchanger is adjusted according to the coolant temperature and is independent of the level of hyperventilation in the range 10 to 100 L/min. The heat exchanger is connected to the refrigerator by insulated tubes. For direction of airflow the system has in- and exhalation valves. Air is fed into a target balloon (15 or 30 L), then through a one-way valve into the heat exchanger. Exhaled air goes through another one-way valve back into the room. The target balloon is continuously filled with dry atmospheric air supplied from the hospital system mixed with 5% CO<sub>2</sub> supplied from a cylinder with pressurized 100% CO<sub>2</sub> in order to produce the eucapnic gas mixture for hyperventilation. Failure to deliver a eucapnic gas would result in hypo- or hypercapnea with potential risk of seizures or severe headache, respectively. The target balloon and its inflation and deflation serves as an optional extra visual control of the hyperventilation rate, while flow through the system is measured by the use of two rotameters, one for atmospheric air with a flow range from 10 to 100 L/min and another for CO<sub>2</sub> in the range from 1 to 10 L/min. The system has a pneumatic control system with time switch which allows preset automatic control of duration of air supply into the system.

#### Dry Air Challenge (DACH)

The system used for hyperventilation with dry air was built by medico-technicians according to an old description (116) and was much simpler than the RHES (7) Figure 3.3.2.b. A commercially available tank with 5% CO<sub>2</sub> enriched, calibrated gas mixture (AGA Gas, Stockholm, Sweden) delivered dry air via one flow meter and

forward via a 15 L target balloon and a large bore tube with a length of ~1.5 meter to the face-mask.

#### Hardware and software for motivation of hyperventilation in CACH & DACH

Bronchial challenge, like lung function testing, has shortcomings in young children. It was soon recognized that young children were unlikely to comply with the conventional protocol for CACH in school children simply relying on watching and keeping a constant size of the target balloon during 4 min (117). Young children are not motivated by this setup and also have very limited sense of time duration, which made it necessary to have a simple way to help them track time during challenge. Biomedical engineers were therefore engaged to develop a creative tool to overcome these obstacles (1). Ventilation in L/min was measured at the exhalation valve (Hans Rudolph, inc., Kansas City, USA) by a pneumotachograph (Hans Rudolph, inc., Kansas City, USA). The analog signal from the pneumotachograph was converted by an analog-digital converter (Pico Technology Limited, Cambridge, UK). A software program displayed the digital signal on a computer screen as the flying height of an animated air balloon (photo on front page and Figure 3.3.2.a + b.). An adjustable horizontal dashed line on the computer screen reflected the target ventilation rate. The total ventilatory volume was shown on screen during and after ending the provocation. An animation of a sun, which crossed the screen in an adjustable number of minutes was used to help the child track time (1). The second generation software is as of 2005 available for free at [www.copsac.dk](http://www.copsac.dk).

#### Methacholine Challenge (MCh)

Isotonic methacholine chloride solution at 0.5, 1, 2, 4, 8, 16, 32, and 64 g/L delivered by the hospital pharmacy was supplied with two extra solutions of 0.162 and 0.250 g/L prepared on location. The solutions were nebulized with a Wright nebulizer (Clement Clarke, Essex, UK) inside a special designed aerosol-box developed by Klug (24). Instead of a pneumotachograph for the measurement of volume, we used a gas meter.

### Mouth piece/face mask

For all challenge techniques we used the face-mask with a built-in mouthpiece as previously described (3.2.2). The rationale was however somewhat different: the important aspects were to prevent nose breathing by securing mouth breathing, stable access to the airways and preventing inhalation of room air accomplished by the tight seal from the cushions of the mask, i.e. the only source of air supply, whether cold, dry or containing methacholine, to the child was through the mouthpiece.

### 3.3.3 How to challenge and practical aspects

#### Cold Air Challenge (CACH)

##### Patient preparation at inclusion, day(s) before testing

When tests were scheduled, parents were given a list of items/medications to avoid before the test (Table 3.3.3). The table lists medications that can decrease airway responsiveness and the time period for which each should be withheld before the test (115). Furthermore they were told to reschedule appointment if the child had had any respiratory infection within the last 3 weeks and if any acute exacerbations had occurred from exposure to an inhalant allergen within the last week, since both are known to increase responsiveness. Challenge was postponed until all of listed criteria were met. Parents were told that their child could experience some minor symptoms, such as cough or chest tightness, but that most patients have no symptoms until after the testing. They were also warned that occasionally severe symptoms could occur.

##### Introductory maneuvers

The RHES was switched on at least 45 min before a test in order to reach a coolant temperature of approximately  $-18$  to  $-20^{\circ}\text{C}$  necessary for the RHES to be able to deliver the desired  $-15^{\circ}\text{C}$  cold air as measured 10 cm from the mouth (1). The child was evaluated for possible contraindications and medication use was reviewed. The dry air and 100%  $\text{CO}_2$  was mixed from separate flow meters to secure that total airflow was in accordance with the target rate of hyperventilation and contained (volume%) 95% dry air and 5%  $\text{CO}_2$ . This was assured before starting the provocation, i.e. the flow meters measuring the atmospheric air supply and the supply of 100%  $\text{CO}_2$ , respectively, were adjusted and set at the calculated flow rates. The pneumatic control system with time switch and timer secured an instantaneous delivery of the desired gas mixture at the calculated flow and for the desired duration.

The dashed line on the balloon animation program was set to show the target ventilation rate, i.e. to a certain level on the screen corresponding to the hyperventilation rate, which was aimed at 1 liter/min/kg body weight. That is, if a child's weight was 16 kg, the

dashed line was adjusted to the level of exactly 16 Liter/min. First of all, this procedure was for simplification and not based on evidence. However, the rationale for choosing this procedure was in fact relying on assumptions and approximations (1). In school children the appropriate level of voluntary hyperventilation to induce bronchoconstriction is usually calculated as  $25 \times \text{FEV}_1$  per min using the pre-challenge  $\text{FEV}_1$  measurement (118), which is actually considered near maximal voluntary ventilation (MVV) (118), whereas other groups originally chose a level corresponding to 75% of MVV (117) and in adults a level of  $30 \times$  baseline  $\text{FEV}_1$  is chosen as standard (119). It is well known that  $\text{FEV}_1$  is closely and significantly correlated to age, height and weight in school children (98) and young children (105), however with height being most important single determinant (105). Since most young children can not perform reliable  $\text{FEV}_1$  measurements we chose to calculate the required hyperventilation rate from the body weight of the participants estimating that 1 L/min/kg of body weight approximates  $25 \times \text{FEV}_1$  per minute. In a study of hyperventilation testing in 9 asthmatic school children the mean weight was 34 kg and the hyperventilation level causing significant bronchoconstriction was 37 to 39 L/min during testing (120) thus supporting our approximation. However, there are some flaws to this assumption. Most important is the risk of overestimating MVV in an adipose child since  $\text{FEV}_1$  is closer related to height than weight. A more precise method could be to calculate the predicted  $\text{FEV}_1$  according to height from the normal equation presented by Eigen (105) and then calculate the desired level of hyperventilation as  $25 \times \text{FEV}_1$ . Measurement of  $\text{FEV}_1$  is obviously another possibility, but only for decision on hyperventilation and not as response measure, since we do not believe that this is a reliable measurement for this purpose in young children, and no reports have challenged this question as yet. Future research should look into which algorithm to use in the decision of hyperventilation level in an individual young child.

##### Verbal instructions

The child (and parent) was carefully instructed, typically in the following manner: "On the screen you see an air balloon. You are able to take this balloon for a flight by breathing fast into the face-mask. The balloon is to be lifted above the line and you must continue for as long as it takes the sun to cross the screen from this [left] to this [right] side. If the balloon is falling you must breathe faster and you are not finished before the sun has crossed ... we [your mother or father and the investigator] will all cheer for you and help you, if necessary ... if you feel uncomfortable you can always stop ..."

##### Hyperventilation, the test

The test was carried out as a single step 4-min isocapnic hyperventilation test (1, 117). "Single step" to designate the all or none nature of this test in contrast to a multiple step test such as MCh (3.3.1.c) or a multiple step CACH test using increasing levels of hyperventilation at each step – and stepping up until a predetermined change in lung function is reached. The single step test seemed to be the most simple and least time consuming test and was previously shown to be of similar value to multiple steps tests in disclosing BHR in the same individuals (121, 122) That a CACH of "4 min" duration was sufficient was demonstrated in school children by Zach and Polgar (123) suggesting a dose-response interrelationship with a reaction plateau the last 3 min of a 10 min CACH, but with no statistically significant difference in BHR between a 4- and a 10 min CACH test performed 10 days apart (123). However, studies in adults have questioned this observation suggesting that increasing the duration of hyperventilation delays the onset of bronchoconstriction but causes greater bronchoconstriction once the hyperventilation is stopped (124). However, for simplification we chose to be content with the 4-min test.

The young child was carefully instructed according to the above-mentioned. The target balloon was filled up before the child started

**Table 3.3.3.** Medication, items or activity to avoid before bronchial challenge testing.

	Minimum time interval from last "dose" to study
Short-acting $\beta_2$ -agonists: salbutamol, terbutaline	8 h
Medium-acting bronchodilators: ipratropium	24 h
Long-acting $\beta_2$ -agonists: salmeterol, formoterol	48 h
Antihistamines	3 d
Leukotriene receptor antagonists	5 d
Inhaled corticosteroids	No restriction, if dose was unchanged the last month
Oral steroids	1 month
Cola, chocolate	Day of study
Exercise and passive smoking	2 h



to hyperventilate in the face-mask. The balloon (on screen) was very sensitive and reached target level within a couple of seconds, but was also losing height promptly if the child was noncompliant. The investigator was constantly cheering and also secured head and face-mask position to prevent air leakage by a slightly firm grip in the back of the child's head with one hand and the facemask with the other. During the challenge the child was asked to constantly focus on the balloon and to secure that it stayed above the line indicating the hyperventilation level. Any sign of discomfort of the child led to canceling of the challenge.

#### *Timing of lung function measurements post-challenge*

Lung function measurements at baseline were done in duplicates. The bronchial response to hyperventilation tests is steep and short, often completely cleared within 12 minutes after provocation (125) in contrast to exercise induced bronchoconstriction (126). The response measurements were performed in the time interval from 3 to 5 min after end of the CACH, since this period was found to be the optimal response window in school children (118, 123), and confirmed in a pilot study in 14 asthmatic school children (unpublished). Post-challenge sRaw was measured in duplicate beginning 3 min after end of challenge and the child entered the box 45 s before measurement. Rint and IOS, in the mentioned order, was only done in singlet due to the time consumption of each of these tests. All lung function measurements were done by the investigator for reasons emphasized previously (27).

#### **Dry Air Challenge (DACH)**

*Patient preparation at inclusion, day(s) before testing*

Not different from CACH.

#### *Introductory maneuvers*

The system was ready for use whenever needed and the child was evaluated as described for CACH. This test did not change concerns. The flow of prefabricated calibrated gas was adjusted on a single flow meter in accordance with the target rate of hyperventilation. This was assured before starting the provocation, but in this setup we had no pneumatic control system with time switch and timer. The balloon animation program was set as explained for CACH i.e. the target ventilation rate was aimed at 1 liter/min/kg body weight. The dry air system also employed a target balloon as extra guidance of ventilation rate. The instructions to the child were identical to CACH.

#### *Hyperventilation, the test*

The test was adopted and in accordance with recommendations from Argyros and colleagues (119), but with a minor modification of target flow. Thus, our protocol (7) was an uninterrupted single step 6-min eucapnic hyperventilation challenge with dry, room temperature gas with a target minute ventilation of  $25 \times FEV_1$ , corresponding to 1 L/min/kg of body weight, similar to the ventilation rate we employed in CACH (1), the modification being the approximation to a ventilation rate of  $25 \times FEV_1$  instead of  $30 \times FEV_1$ . Thus, compared to the CACH test, there were two changes, the duration of hyperventilation and the temperature. The longer duration of hyperventilation was accepted to offer full credit to the test in a comparison with CACH. Indeed we expected DACH to be at least equal to CACH and if more potent the test would seem even more attractive for testing in young children. In adult asthmatics, DACH had demonstrated higher sensitivity than CACH at 100% specificity in one study (127) while other studies had found CACH to be slightly superior to DACH (108, 128, 129).

#### *Timing of lung function measurements post-challenge*

We employed exactly the same time schedule for response measurement as with CACH, however only sRaw was measured in this particular study comparing the two tests. We do appreciate that this

time schedule may have wrongly put the DACH test in disadvantage since the longer duration of challenge may have postponed the post-challenge peak increase in resistance according to a study suggesting that increasing the duration of hyperventilation delays onset although causing greater bronchoconstriction (124). However, we did not observe signs of this (7).

#### **Methacholine Challenge (MCh)**

*Patient preparation at inclusion, day(s) before testing*

Not different from CACH.

#### *Introductory maneuvers*

The system was ready for use when the PtcO<sub>2</sub> monitor had reached steady state (~20 min). The child was evaluated as described for CACH. This test did not change concerns.

#### *Challenge procedure*

The MCh test was done as a multi-step challenge with a dosimetric method. Methacholine was inhaled by tidal breathing from the aerosol box through the special face-mask with built-in mouthpiece. At each step of the challenge the subject inhaled 200 mL of the aerosol per kg of body weight. Doubling concentrations of methacholine, from 0.0625 to a maximum of 64 mg/mL, were used. Increasing doses were inhaled until sRaw increased by 50%, the maximum methacholine dose of 64 mg/ml was reached, clinical airway obstruction was apparent, the subject complained of discomfort, or PtcO<sub>2</sub>, which was monitored continuously during provocation, exhibited a decrease of  $\geq 3$  kPa (21, 22, 24).

#### *Timing of lung function and PtcO<sub>2</sub> measurements*

Duplicate measurements of Rint, IOS, and sRaw were made at baseline. PtcO<sub>2</sub> was measured continuously until end of session. Duplicate measurements of Rint were made 2 min after each step until Rint increased  $\geq 10\%$  from baseline. At that point sRaw, Rrs<sub>5</sub>, or Xrs<sub>5</sub> were measured in the mentioned sequence. The response to a challenge was measured from 3 to 5 min after the end of the challenge. PtcO<sub>2</sub> was read as mentioned earlier (3.2.4).

#### **3.3.4 Quality control of challenges**

Before challenges parents were once again asked about the child's condition and actual medication according to (3.3.3). Any deviation from the protocol or symptoms indicating that the child was not well resulted in postponing the test until all criteria were met.

No CACH was started unless RHES temperature was at least  $-18^\circ\text{C}$ .

CACH and DACH of shorter duration than 4 min and 6 min, respectively, were not accepted. A challenge was always accepted if hyperventilation (level of balloon) was above the desired level during all 4 and 6 minutes. If the level was fluctuating around, but predominantly above the level, the challenge was also considered sufficient. If the level fluctuated, but predominantly below the desired level although close and the child completed all 4 minutes, the test was accepted at the discretion of the investigator. A test was however not accepted if the level was predominantly below the desired level during the first 1 to 2 min, which always caused canceling of the test. The new version of the software (3.3.2) has optional facilities for improvement of quality control of the challenge test. A MCh step was only considered successful if the desired volume of aerosol was inhaled, i.e. a child weighing 15 kg should be able to inhale at least 3 L at each step. Quality control is an issue not addressed in reviews of or guidelines for bronchial challenge procedures (29, 30, 115).

#### **3.3.5 Repeatability**

##### *CACH and DACH*

Short term repeatability within minutes is not applicable to challenge procedures due to refractoriness as demonstrated in the comparison of CACH and DACH (7). However, despite possible refractoriness after CACH (7), a cautious comparison between DACH and

CACH performed with an interval of approximately 1 hour in 40 young children showed a significant, but weak correlation ( $r^2=0.34$ ,  $p<0.0001$ ) between responsiveness to the 2 challenges. A Bland-Altman plot of difference between CACH and DACH in sRaw response calculated as numbers of SDw-units versus the mean change in SDw-units after CACH and DACH, respectively, gave a mean difference of 1.8 SDw or 9% in favor of CACH and limits of agreement of  $-8$  SDw and 12 SDw.

Long term repeatability was investigated twice with an interval of 8 weeks in 13 asthmatic children with BHR to CACH (positive), defined as a response of  $>3$ SDw as measured by sRaw. The second CACH test was positive in 11/13 (85%), the correlation coefficient (95% CI) was 96% (87-99%) ( $p<0.0001$ ) (1). Rint and IOS exhibited poor repeatability. Likewise ICC between screening visit and inclusion (2 months) was 0.66 and between inclusion and final visit (8 weeks ICS, DBPC trial) 0.83 in the placebo group (3). Again, CACH as measured with Rint and IOS showed very poor repeatability. We did not perform studies on repeatability between observers.

### MCh

Dosimetry was used since this was recommended to reduce the variability of the dose of the drug to the lungs (24). Data on repeatability of Mch employing multiple comparisons of sRaw, IOS, Rint and PtcO<sub>2</sub> as assessments of BR showed dependency on the lung function technique (24). The day-to-day repeatability studied in 8 to 16 young children (different success in obtaining PD%) showed that the reactivity on average could be repeated within one doubling dose (dd) for Xrs<sub>5</sub> (0.75 dd), PtcO<sub>2</sub> (0.49 to 0.59 dd), and sRaw (0.72 dd), the latter comparable to that reported in school children and adults (24), whereas Rint (1.19 dd) and Rrs<sub>5</sub> (1.64 dd) showed poor repeatability. Evaluating the repeatability of PD% using SDw came out with exactly the same ranking of estimates (24) The ICC was higher than 0.6 for all of the estimates, except for PD30%Rrs<sub>5</sub>. Repeatability was not related to the magnitude of BR. Another study using sRaw showed good correlation ( $r=0.97$ ) between BR (PC100%His) measured on 2 consecutive days in 13 preschool children (130). The reproducibility of sRaw, PD100, and BR, using carbachol challenges twice within 7 days in 20 healthy and 20 hyperresponsive 3 to 6 year-old children showed a coefficient of repeatability for PD100sRaw of 3.9  $\mu$ g (42). A study using FOT as response found satisfactory 24 hour interval within-subject reproducibility of threshold dose and provocative dose to histamine in asthmatic children 3.9 to 8.5 years) (34). A study of Mch repeatability in 10 five year old asthmatic children compared 4 different Rint estimates, Rrs by FOT and PtcO<sub>2</sub> (40). PtcO<sub>2</sub> had the lowest variability. MCh measured with PtcO<sub>2</sub> in eight 2 to 5 year old children on separate days during sleep showed good correlation ( $r=0.94$ ) (35). In a population based follow-up study of natural history of wheeze and BR using MCh and PtcO<sub>2</sub> an ICC of 0.74 was found in a subgroup of 30 preschool children, measured twice 9 months (1-20 mo) apart (131).

### 3.3.6 Normative data

We were first and hitherto the only group to report on responsiveness to CACH in 2 to 5 year old healthy and asthmatic children (1). We studied CACH responsiveness by whole-body plethysmography, interrupter technique and IOS. Results are given in Table 4.1.2.a. In contrast to CACH, DACH has not been applied in healthy young children for comparison with asthmatics, but our results (7) suggest that DACH may discriminate between health and disease with an equal sensitivity, however this has to be properly addressed in a future study. A 60% increase in sRaw was suggested as the upper normal level of responsiveness to a DACH in non-asthmatics in a study employing sRaw measurements as response (47). However, such a large normal increase is probably an overestimation, since it corresponds to an increase of approximately 6 to 7 SDw in our setup and thus far beyond our cut-off at 3 SDw. The literature does not pro-

vide any normative data on MCh responsiveness in healthy young children.

### 3.3.7 Acceptance

The general experience from all challenge studies was that if a child was able to perform the different lung function tests the child was also likely to perform satisfactory challenge procedures (1-4, 6, 7) In study I (1) CACH was attempted in 71 children and completed in 67 (94%), 29 healthy and 38 asthmatic young children. Four 2 year old children in the group of healthy children were not able to hyperventilate cold air at the desired level, although they did perform satisfactory lung function tests. All asthmatic children completed the test (1). In the study comparing DACH with CACH (7) we did not observe any difference in acceptability despite 50% longer duration of provocation, appreciating that all participants were well acquainted with the procedures. However, it is likely that acceptability of DACH will drop considerably in very young children due to a longer duration of challenge, once again favoring CACH, unless a 4 min single step DACH is proven equally sensitive. A recent publication from a large cohort study (47) reported acceptance of DACH in 526/690 (76%) five year old children using the original protocol for CACH in school children (117). This is a surprisingly low success rate considering that the children were all five years old and the challenge duration was only 4 minutes, emphasizing the need for the "balloon-software" that we have developed.

MCh in particular was generally well accepted and tolerated, whereas problems arose related to acceptability of the endurance that was expected from the young child during the often more than 1 hour sessions when performing all the different tests (22) and particularly the local skin irritation from PtcO<sub>2</sub>-electrode gave minor problems in 5 of 39 (13%) patients in one study as previously mentioned. Interestingly, MCh was successfully completed in only 35 of 50 (70%) three year-old children (36).

### 3.3.8 Safety

We did not encounter one single serious complication in more than 600 CACH and 80 DACH procedures in young children. In those children disclosing a positive and clinical reaction, the duration was brief and transient and only a couple of children needed rescue medication. Studies in school children and reports from departments performing CACH as part of daily routine also reported no serious complications among 2,000 children though approximately 80% of the tests were done in patients with an established diagnosis of asthma. (121, 122) Furthermore CACH does not result in late phase asthmatic reactions (132). MCh using PtcO<sub>2</sub> is considered safe in young children (41). Despite these reassuring data on safety we always follow basic safety rules for bronchial challenge procedures: 1) Two experienced people in attendance, one is a pediatrician. 2) Ventilation is measured since this is the stimulus. 3) Bronchodilator plus oxygen and a pulse oximeter at hand. 4) Medical help/resuscitation available within 2 minutes 5) Cessation of test if the child appears distressed or breathing is labored.

Preceding all the studies (1-4, 6, 7) in young children, we did a pilot study in 8 children, mean age 4.5 years and mean weight 18.3 kg, in which PtcCO<sub>2</sub> was monitored during a CACH (1). The mean values of PtcCO<sub>2</sub> were calculated from four readings at one minute intervals of the hard-copy curves printed during measurements, before and during CACH, respectively. Mean baseline PtcCO<sub>2</sub> was 4.8 kPa and mean PtcCO<sub>2</sub> during CACH test was 5.1 kPa, i.e. a mean (range) increase in PtcCO<sub>2</sub> of no more than 0.3 (0-0.5) kPa. PtcCO<sub>2</sub> did not decrease in any of the children. Our method of hyperventilation of air mixed with 5% CO<sub>2</sub> in this age group was therefore considered safe and no further monitoring of blood gases was found necessary.

### 3.4 STATISTICS

Central tendency was expressed by the mean and 95% confidence

interval (CI) of the mean and the boundaries of values in groups were described by SD, range or confidence limits, when data were normally distributed. Otherwise median and range or percentiles were given.

The SDw was calculated as the SD of differences between paired baseline measurements from all participants divided by  $\sqrt{2}$ . SDw may be used as a descriptor of repeatability, but was primarily used in calculations of the sensitivity index (see below) (1, 3, 5-7).

“Sensitivity” may be defined in two slightly different ways:

1. *Sensitivity to detect changes* in e.g. lung function: here *sensitivity* refers to the power of a certain measurement method to exhibit a change in this measure. The sensitivity of a test cannot be assessed without the knowledge of its repeatability expressed e.g. as the SDw. A change in a measurement value of more than 2 SDw is thus considered a true change to e.g. a stimulus because it is outside the range of values likely to occur just by repeating the test at baseline. The sensitivity of a test to detect e.g. a change due to CACH can then be described by the sensitivity index calculated from the formula:

Sensitivity index = (post CACH value – baseline value)/SDw,

giving the change as number of SDw's, sometimes referred to as SDw-units. The sensitivity index allows for direct comparison between different methods because this value is independent of the measurement scale and accounts for differences in reproducibility or repeatability (1, 5, 7, 96).

In study I (1) the sensitivity of each of the lung function tests to detect changes after CACH could be ranked by their mean changes calculated as number of SDw-units: sRaw (9 SDw-units) > Rint (2.5 SDw-units) > Xrs<sub>5</sub> (1.8 SDw-units) > Rrs<sub>5</sub> (1.4 SDw-units), Table 4.1.2.a.

2. *Sensitivity to detect disease*: Here, the *sensitivity* is the probability that a test result will be positive when the disease is present (also described as the true positive rate). In order to be able to determine this value of sensitivity it is necessary to know how the different methods respond when testing is performed in healthy children. Using a simple 2 × 2 table for a given cut-off value, for all tests, makes it possible to calculate comparable sensitivity, specificity and predictive values. However, a further refinement employs the Receiver Operating Characteristic (ROC) curve (a graph that plots the true positive rate in function of the false positive rate, 1 – specificity, at different cut-off points) analysis by which it is possible to identify the optimal cut-off point that discriminates most efficiently between the absence or presence of disease (5).

*Z-score* identifies the distance of an original (raw) score from the mean. The distance is measured in SDb units, e.g., a z-score of 1.0 indicates that the raw score was one standard deviation greater than the mean predicted value. Z-scores are corrected for influential demographic factors and therefore specifically useful for longitudinal studies (6).

Two-tailed paired or unpaired *t-test* was used to assess differences in values within and between groups, when data were normally distributed (1, 2, 4, 5). Two-tailed paired and unpaired *Wilcoxon-test* was used for non-parametric data (7).

*Linear regression analysis* was used to predict one variable from another (5).

*Fisher's exact test* measures the likelihood that the proportion in two independent groups could have happened by chance if the two groups were sampled from the same population (3).

Repeatability was evaluated according to *Bland and Altman* (133), (1, 3, 6, 7) by *Pearson's* linear correlation (1, 3, 6) and by calculations of the *Intraclass Correlation Coefficients* (ICC). ICC is the percentage of the total variation that is accounted for by the between-patient SD (SDb):  $ICC = 100 \cdot SDb^2 / (SDb^2 + SDw^2)$ , since total variation

also includes the variation within patients (SDw). If ICC is >0.6 a test may be considered clinically useful (3, 7).

An *analysis of variance* (ANOVA) model was applied for comparisons of treatments using baseline values as covariates. Also a *multivariate analysis of variance* (MANOVA) model was applied (3).

*Linear Mixed Effects Models* (LINMEM) are used in the evaluation of longitudinal data exhibiting heteroscedasticity (unequal variances) and dependence, which call for structured covariance models (6).

*Wilk's likelihood ratio test* on 1 degree of freedom was used to test the global treatment effect (3).

*Spearman correlation*, rho, was used to test for change in z-score during the study (first and last measured value for each patient) for each method (6). A perfect fit, rho equal to 1, indicates that the patients come in the same order at the first and last visits in the study. However, this does not indicate whether patient's values are unchanged or not. To test for this, Wilcoxon test was performed (see above) (6).

A p value less than 5% was considered statistically significant (1-7).

## 4. AREAS FOR CLINICAL APPLICATION

### 4.1 ASTHMA

#### 4.1.1 Discrimination between health and disease

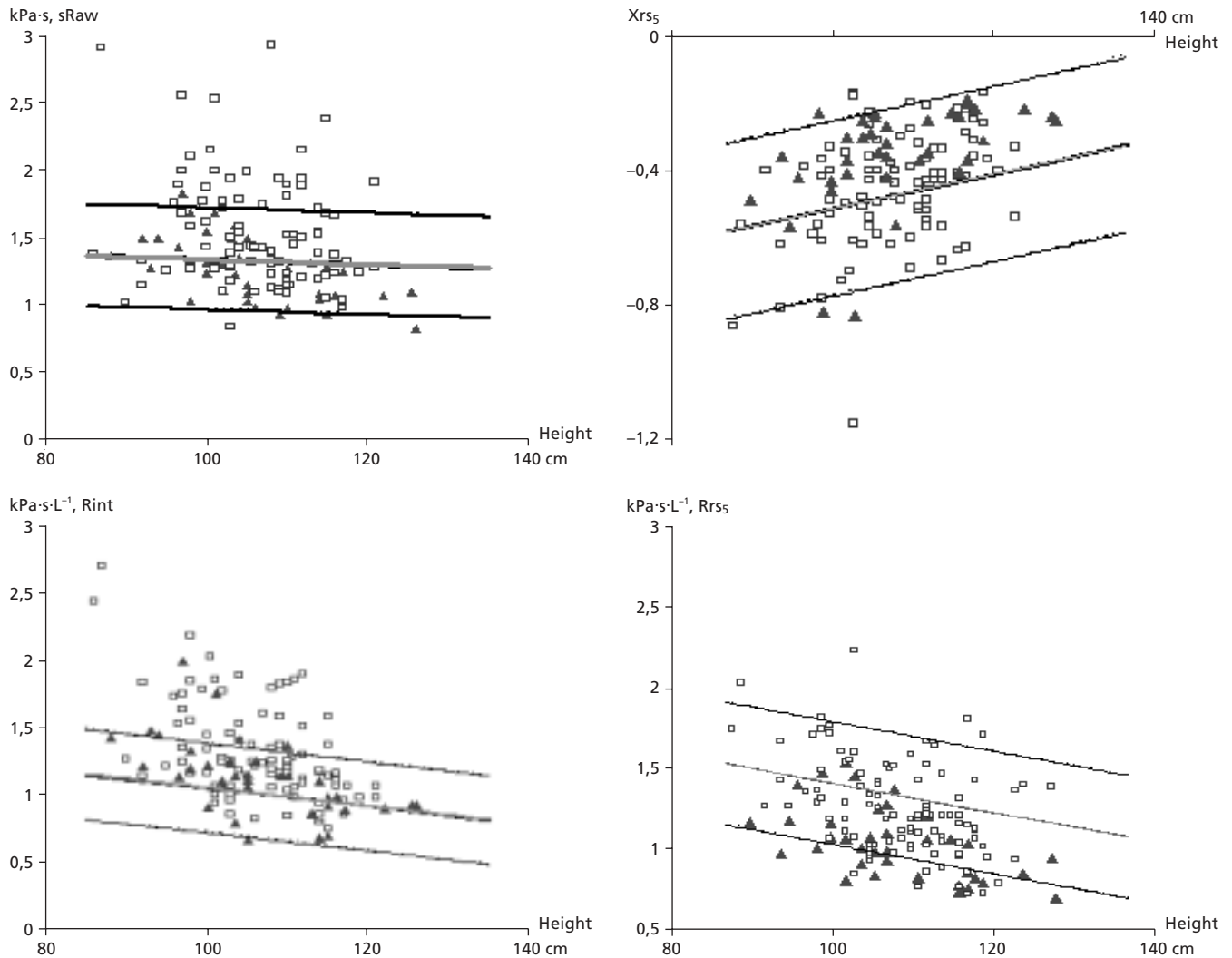
##### *Whole-body plethysmography*

Mean percent predicted sRaw was statistically significantly increased in selected groups of 2-6 year old asthmatics compared to healthy controls (1-5, 7). In Figure 4.1.1 the pooled data from study I (1) & V (5) are shown.

Mean predicted sRaw had been previously found increased in a random group of asthmatic young children (26) and 5 to 8 year old children (53), in which it was suggested that sRaw may be as efficient as Raw and TGV in distinguishing between asthmatics and healthy. In fact sRaw measurements discriminated more accurately between healthy and asthmatics than Raw measurements (53). In a 4-year prospective study in 129 children with  $\geq 3$  wheezing episodes before age 2, persistent wheezers were found to have statistically significantly higher sRaw after 4 years than those who became asymptomatic (134). sRaw was satisfactory measured with a setup identical to ours in 503/803 (63%) 3 year old children in a large prospective cohort study (54). sRaw was significantly higher in those who wheezed at least once during the first 3 years of life than in those who had never wheezed. Within the latter group there were significant differences in sRaw between different risk groups. sRaw was also significantly related to atopic status and within the non-atopic group sRaw related significantly to risk group (54). 186 (40.9%) of the parents reported their child wheezing in the first three years of life, and this was confirmed in 130 (28.6%) exhibiting significantly higher sRaw than those with unconfirmed wheeze or no history of wheeze (55). In contrast sRaw using approximately identical setup to ours did not discriminate between 44 children with clinical asthma, 44 children with chronic cough, 38 children with wheezy bronchitis in the first 2 years of life and 40 controls (42). All groups showed mean values within the reference range, a reference material from another laboratory. This may point to an extremely important influence from selection bias among healthy and asthmatics and from choice of reference material. In fact, it may be argued that our studies were biased by selection of supernormal children (no previous pulmonary disease and no passive smoking) and hospital dependent asthmatics. Furthermore Badier et al (42) used standard face-mask in some children and mouth piece in others making interpretation of their results even more difficult.

##### *Interrupter technique*

Rint was significantly increased compared to healthy controls in a random group (48 of 109 patients had abnormal values) (26) and in selected groups of 2 to 6 year old asthmatics (1, 3, 5). Data from study I and V are shown in Figure 4.1.1. Both expiratory and inspir-



**Figure 4.1.1.** Baseline sRaw, Rint, Xrs<sub>5</sub>, and Rrs<sub>5</sub> in relation to height. Absolute values in healthy and asthmatic children (data pooled from study I and V). The *middle line* in each graph represents the predicted mean value, and the *parallel upper and lower lines* represent the upper and lower 95% predicted levels. Asthmatic subjects *open squares*; healthy subjects *closed triangles*.

atory Rint were found to be clinically useful to assess airway obstruction in children with asthma or CF as young as 3 years of age and correlated positively with Raw, FEV<sub>1</sub> and FEF<sub>25-75</sub> (81). However, only 12 children were <6 year and no real age-matched normal material was presented. Expiratory Rint was significantly higher in 3-18 year old asthmatics compared to an age-matched healthy control group, however, this observation was hampered by a significantly higher mean age of the latter group (74). Median baseline Rint in preschool children was significantly higher in wheezers than in either coughers or control subjects and coughers did not differ significantly from control subjects (63). Rint showed a significant correlation with spirometry in 42 asthmatics and 125 healthy controls of 0.8 to 16.8 years, but baseline values did not discriminate healthy from asthmatic children (95). A multicenter study compared expiratory Rint in 74 preschool asthmatic children and 84 healthy control subjects (65). Resistance was significantly higher in asthmatics than controls and even more marked in children with symptoms on exertion (65). Children with persistent wheeze had significantly higher Rint than children who never wheezed according to a cohort study (135). Preschool children exhibited significantly increased Rint if the parents currently smoked  $\geq 3$  cigarettes a day in the presence of their child (136). This study was lacking data on parents smoking habits during and after pregnancy, which will be provided in the COPSAC study (43). Furthermore these results points to the importance of passive smoking when choosing reference material, indeed our control subjects were not allowed to have

passive smoking at home, rendering our control subjects supernormal for this marker (1, 25).

#### Impulse Oscillometry

In selected groups of 2 to 6 year old asthmatics the group mean values were statistically significantly outside normal range, however only very few individuals were outside normal reference interval as shown in Figure 4.1.1, (1, 5) and in one study mean values were not statistically different between asthmatics and healthy controls (3). The mean values of Xrs<sub>5</sub> and Rrs<sub>5</sub> were outside normal range in a random group of asthmatic children (26), however, as seen in our studies, only a minority had abnormal values and it is uncertain if the group as such was statistically significantly different from healthy in these parameters.

FOT demonstrated values outside the normal range in 16 of 17 highly selected children with different obstructive airway diseases and abnormal FEV<sub>1</sub>. Reactance was somewhat less discriminative than Rrs at all frequencies (137). In 281 preschool children baseline values of IOS were not significantly different between healthy and stable asthmatics (88). IOS in 73 4-year-old children using our setup showed no significant differences in resistance or reactance at 5 or 10 Hz or resonant frequency between the 28 children with or the 45 without asthma (72). IOS, both Rrs<sub>5</sub> and Xrs<sub>5</sub>, discriminated significantly between 62 healthy preschool children and 50 children with probable or treated asthma (138). 46 children with chronic cough did not differ from healthy in lung function. NO seemed superior to

baseline IOS and BDR as measured with IOS in discriminative capacity (138).

In conclusion, these studies report overwhelming evidence that

IOS has very poor discriminative power at baseline as compared to sRaw and Rint.

#### 4.1.2 Bronchial challenge and responsiveness

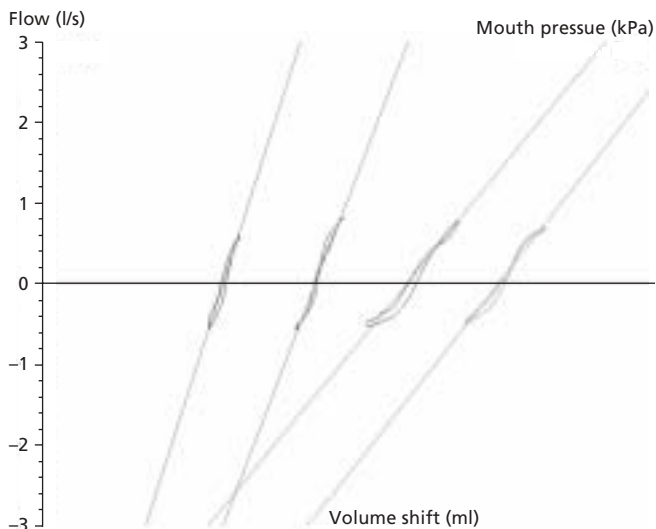
##### Cold Air Challenge in Young Children

Because exposure to cold, dry air relates more closely to the pathophysiology of asthma than does pharmacological provocation, we established a method of CACH and assessed the feasibility in young children. We were the first and hitherto the only group to report responsiveness to CACH in 2 to 5 year old children (1). We studied CACH responsiveness in asthmatics and healthy young children (1) and were therefore able to judge the discriminative capacity of CACH. Since each of the lung function measures, whole-body plethysmography, interrupter technique and impulse oscillometry were applied, we were also able to judge the discriminative capacity of each test.

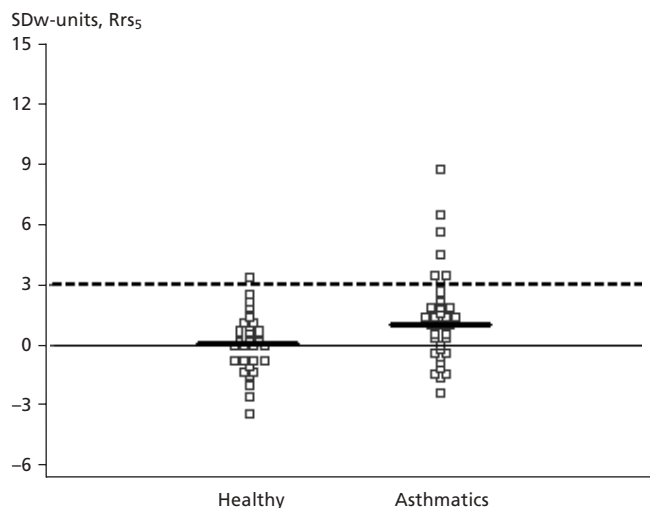
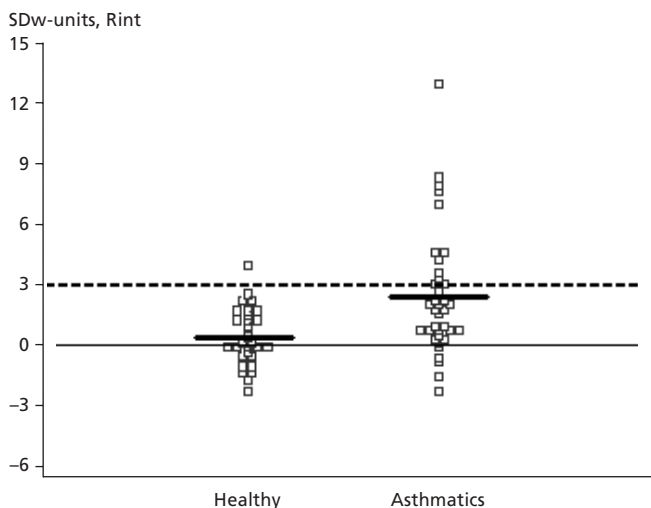
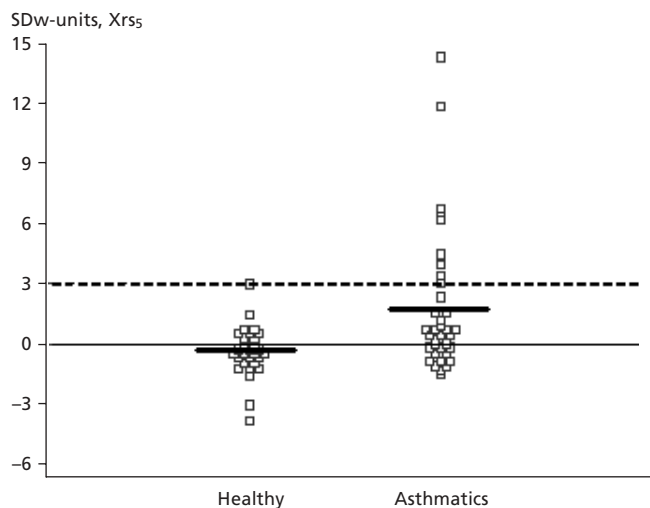
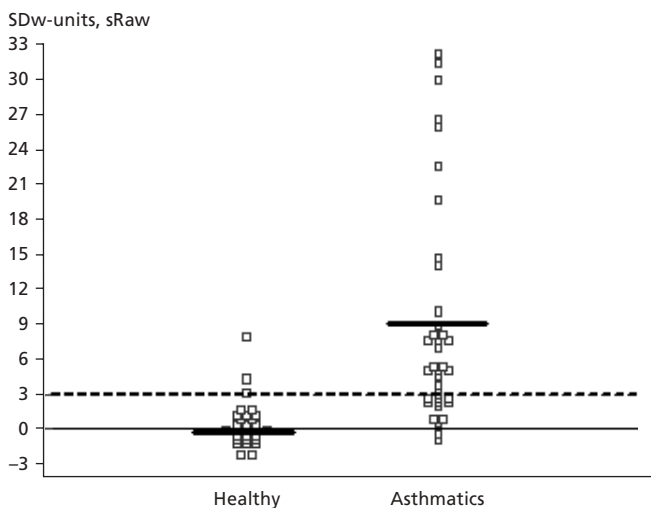
The challenge was readily performed in 38 asthmatic and 29 healthy children aged 2 to 5 years. CACH was feasible and showed good acceptability and did not raise any concerns about safety. A representative example of a result of CACH in an individual young child with asthma as measured with whole-body plethysmography is shown in Figure 4.1.2.a.

Results presented as change in lung function calculated as SDw-units are shown in Figure 4.1.2.b and given in Table 4.1.2.a.

We appreciate that responsiveness measured with sRaw was not normally distributed and therefore also provide the data on medians (not published). The differences in responsiveness between healthy controls and asthmatics were highly statistically significant, however with some overlap as expected from reports on CACH (117, 118,



**Figure 4.1.2.a.** An example of test results from a CACH. Two specific resistance loops at baseline as measured with whole-body plethysmography are seen at the left (each loop is a representative of 5 loops) and likewise two loops from measurements after CACH are seen at the right. Notice parallel parameter lines in each set of measures as evidence of good repeatability. In this example the sRaw was increased by 300% as reflected by narrowing of the closed angle  $\beta$  between the x-axis and the parameter line (see formula in 3.2.3).



**Figure 4.1.2.b.** CACH induced changes in lung function measurements (sRaw, Rint, Xrs<sub>5</sub>, and Rrs<sub>5</sub>) given as number of SDw-units for asthmatics and healthy young children. Means are indicated by horizontal lines. Dotted line indicates the 3 SDw change limit.

**Table 4.1.2.a.** Changes in lung function after cold air challenge in healthy and asthmatic young children: number of SDw-units [mean (95%CI), median (95%CI) and range].

Lung function test	Healthy			Asthmatics		
	Mean	median	range	Mean	median	range
sRaw	0.6 (-0.2; 1.3)	0.1 (-0.5; 1.0)	-2.1-8.0	9.0 (5.9; 12.1)	5.4 (3.0; 8.1)	-0.9-32.1
Rint	0.5 (-0.1; 1.0)	0.2 (-0.3; 1.4)	-2.2-4.0	2.5 (1.5; 3.5)	2.0 (0.9; 2.7)	-2.2-13.0
Rrs <sub>5</sub>	0.1 (-0.5; 0.6)	0.1 (-0.7; 0.8)	-3.5-3.4	1.4 (0.6; 2.2)	1.4 (0.3; 1.9)	-2.4-8.7
Xrs <sub>5</sub>	0.3 (-0.2; 0.8)	0.3 (-0.2; 0.6)	-3.0-3.9	1.8 (0.6; 2.9)	0.6 (0.0; 1.4)	-1.5-14.0

**Table 4.1.2.b.** Sensitivity, specificity, and predictive values of each lung function test using change > 3 SDw-units after CACH as definition of bronchial hyperresponsiveness.

Lung function test	Sensitivity (%)	Specificity (%)	Predictive value of positive test (%)	Predictive value of negative test (%)
sRaw	68	93	93	69
Rint	32	97	92	52
Xrs <sub>5</sub>	24	100	100	50
Rrs <sub>5</sub>	19	97	88	47

125) and MCh (139) in school children. In fact one healthy child exhibited BHR of 8 SDw which was the reason for the relatively wide range of responses in healthy as measured with sRaw.

BHR, defined as an increase in resistance measure of more than 3 SDw was considered relevant and permitted for comparison of different lung function techniques. This occurred in 68% of the asthmatic children and 7% of the controls when measured by sRaw. Hyperresponsiveness was detected in 32% of the asthmatic children by Rint, in 24% by measurement of Xrs<sub>5</sub>, and in 19% by measuring Rrs<sub>5</sub> attesting to the superiority of sRaw for detection of BHR in asthmatics (1). Table 4.1.2.b gives relevant figures for the discriminative capacity of each test. Although all tests demonstrated high specificities, only sRaw exhibited reasonable capacity to function as a diagnostic test in combination with CACH.

Three SDw-units corresponds to a cut-off at ~20% increase in sRaw. However, a more correct method would be to employ calculation of ROC-curves as performed in the study of BDR (5).

#### Dry Air Challenge in young children

Having demonstrated the usefulness of CACH we were interested in developing an even simpler hyperventilation technique. Not only is the RHES equipment for CACH approximately 10 times more expensive than the equipment for DACH in initial costs and service expenditures, it also takes a lot of space and more time for preparation before testing can begin. Modern RHES equipment is however both much smaller and faster (ready in 15 min). The dry-air is simply obtained from a commercially available tank containing compressed

air ready for eucapnic hyperventilation. Such simplified set-up could ease the dissemination of indirect challenge test for BHR in young children. For these reasons we compared responsiveness in 40 2- to 5-year-old asthmatics between two standardized, single step protocols: DACH performed as 6-min eucapnic hyperventilation with dry room temperature air, and CACH as 4-min hyperventilation (3.3.1.1 & 3.3.1.2) Response was measured as sRaw and expressed as change from baseline in SDw-units. Challenge sequence was randomly assigned. A comparator challenge was performed ≥ 1 hour later if the first challenge gave a change of ≥ 3 SDw-units (7).

Responsiveness to CACH versus DACH showed significant, but weak correlation ( $r^2=0.34$ ,  $p<0.0001$ ), though responsiveness to CACH exceeded DACH (7.6 vs. 5.4 SDw-units,  $p<0.02$ ). CACH seemed to induce reduction in response to the following DACH ( $p<0.01$ ), while no such reduction was seen after DACH. Despite this statistically significant difference in the mean responsiveness and the suggested refractoriness after CACH, 85% of the patients were positive with both tests as demonstrated by an increase in sRaw of ≥ 3 SDw-units corresponding to ~20% or more (7).

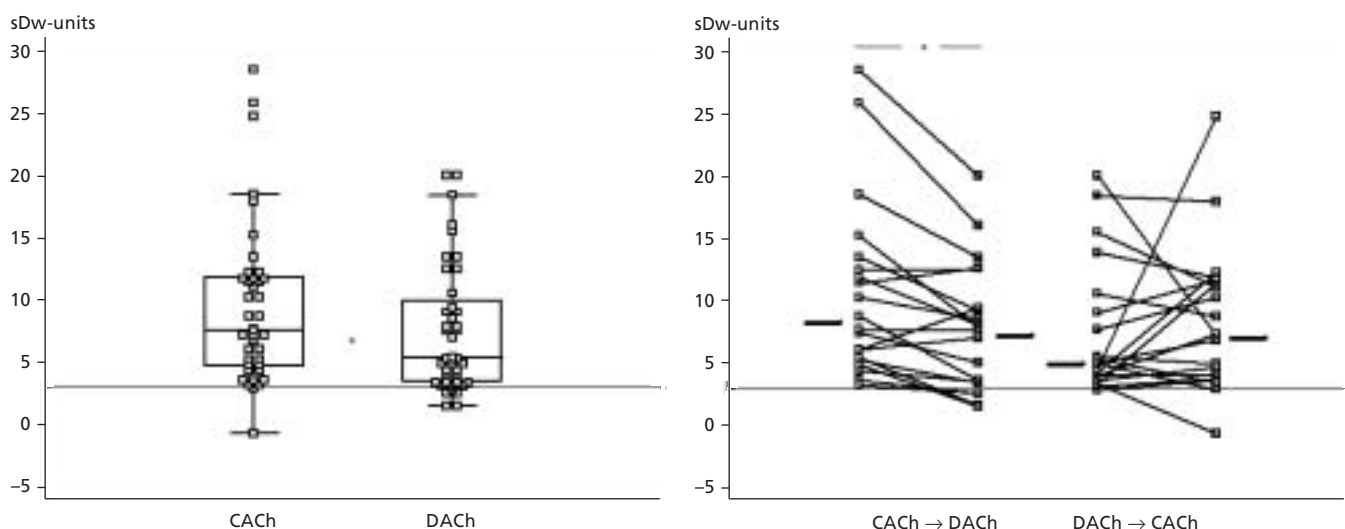
Recently we were encouraged by the publication from Lowe and colleagues reporting a large cohort study successfully using DACH and sRaw in 526 five year old children (47).

#### Asthma and bronchial responsiveness

The chronically inflamed airways in asthma become obstructed and airflow limited by bronchoconstriction, mucus plugging and increased inflammation when the airways are exposed to various triggers and results in wheezing, breathlessness and coughing. These naturally occurring disease events of asthma may be imitated in the laboratory by various methodologies. Basically these methods rest on the stimulus-response concept of asthma, by which different triggers (stimuli) are applied to the airways, and the response, particularly the bronchoconstriction, is measured.

#### Definitions

Bronchial responsiveness (BR) is merely expressing the measured change to a stimulus in any given measurement related to the airways in the asthmatic patient. In contrast bronchial hyperrespon-



**Figure 4.1.2.c.** Left: Responsiveness given as SDw-units to CACH and DACH with ~1 hour interval in young children. Right: Same data but broken into subsets according to order of challenge modality, CACH to DACH or DACH to CACH. Notice significant reduction in responsiveness from CACH to DACH. \*)  $p<0.05$ .

siveness (BHR) is by definition an abnormal change in this measurement exceeding what is seen in healthy subjects. In fact, BHR is a characteristic and major pathophysiological phenomenon of asthma (32), defined as an abnormal increase in airflow limitation following exposure to a non-allergenic stimulus (29-31). The abnormal increase is for clinical and practical purposes often defined by a cut-off level beyond which a certain response is deemed abnormal. This cut-off is conventionally equal to or higher than 2SDw of the method but should preferably be determined from statistical calculations involving ROC curve analyzes (3.4).

The notion that a patient exhibits symptoms of BHR is often used by the clinician to describe the history taken from the patient, but does not necessarily reflect that the patient will exhibit any BHR as defined above, when challenged in the laboratory. Unfortunately these two interpretations of BHR are used interchangeably leading to some confusion.

The different types of stimuli, direct and indirect have been previously defined and described (3.3). Methods for assessment of airflow limitation may also be divided into direct e.g. various lung function tests and indirect tests exemplified by auscultation and measurement of PtcO<sub>2</sub>.

#### *Do young children exhibit clinical signs of BHR?*

Just like anyone else with asthma, young asthmatic children often exhibit symptoms which are thought to be typical signs of BHR. BHR should be considered in all young children with suspected or established asthma and all young children with a history of cough, shortness of breath or wheezing associated with playing, exercising, shouting or crying. Parents should be questioned about symptoms following exertion. It is important to establish that children who avoid vigorous activity are not so constrained by disagreeable experiences deriving from asthmatic symptoms arising during playing and exertion. Such symptoms however often render pediatricians to feel comfortable with the diagnosis (140). The clinician is however always left with an agonizing doubt to the question: is it really asthma and was differential diagnoses ruled out? Objective measures of lung function and documentation of BHR may help to achieve a more certain diagnosis and to design a more suitable treatment for the individual child. The fact that young children with asthma exhibit BHR as part of their disease has been documented in a limited number of studies as referenced below and specifically studies comparing with BR in healthy are lacking. Young children with asthmatic symptoms seem to represent a very heterogeneous group of patients and are therefore much more difficult to encompass into the universal definition of asthma as a chronic inflammatory airway disease exhibiting BHR as part of the picture. The asthma diagnosis is foremost completely based on history-taking and second hand clinical observations and most decisions on diagnosis and treatment are therefore heavily dependent on observations by the parents. Furthermore, according to GINA guidelines (32), decisions on treatment strategy with ICS are entirely based on symptoms and diagnostic clinical algorithms. Therefore we are obviously in great need of a simple, clinically useful, objective method to help demonstrate probable or improbable existence of airway inflammation in a young child with asthmatic symptoms. Such information has been achieved from bronchial challenges in school children and adults for many years rendering correct diagnosis much safer, whereas testing for diagnostic purposes has been nonexistent in young children.

A simple test to separate patients with BHR from normo-responsive patients is therefore needed. Not only will demonstration of BHR strengthen the asthma diagnosis, which is important for clinical practice, but regular (e.g. yearly) assessment of BR will also provide means to adjust and optimize treatment, since it is known from studies in adults that BHR is related to asthma severity and airway inflammation (141). It has been reported that a treatment protocol aimed at improving BHR to methacholine, as well as symptoms and

lung function, led to better asthma control, fewer exacerbations and reduced chronic airway inflammation in adults (142). The measurement of BR will also provide the mean for more specific clinical trials of asthma treatment in young children.

#### *Why not solely rely on history and physical examination?*

History and physical examination do not detect BHR with sufficient accuracy as shown in school children and adolescents (143). A screening history suggested exercise induced asthma (EIA) in 39.5% of children, but only 12.9% actually had documented EIA. Moreover, EIA was diagnosed in 7.8% of the subjects with negative questionnaires and physical examinations, while a similar proportion with no previous diagnosis of EIA, asthma, or allergic rhinitis had EIA diagnosed by exercise challenge (143). There are for obvious reasons no data to support such observations in young children at present.

#### *Why assess bronchial responsiveness?*

Demonstration of BHR is useful in diagnosis and characterization of children with respiratory disease and nearly all children with asthma exhibit some degree of BHR, which like the asthma itself, fluctuates over time and is more pronounced during symptomatic episodes (32). Furthermore, the severity of BHR as determined from direct tests seems to predict the response to ICS in adults (144), though this is less convincing in school children (145). However, such predictions may be more pronounced with indirect tests since they seem to have a high sensitivity for identifying asthmatic patients who have the potential to respond to anti-inflammatory treatment (31).

#### *BHR as predictor of asthma or ongoing asthma*

Because of evidence that abnormalities early in life are important in causing childhood asthma, Palmer and colleagues (12) determined whether BHR at one month predicts asthma at age 6. BHR to histamine at one month was associated with decreases in FEV<sub>1</sub> and FVC and with increases in physician-diagnosed asthma and lower respiratory tract symptoms at age 6. They concluded that BHR at one month predicts abnormal lung function and the emergence of asthma by 6 years of age. In another study the role of BHR as a determinant of persistence of wheezing from infancy to school age was assessed (134). In a follow-up study, 129 infants with  $\geq 3$  episodes of wheezing before age 2 were followed during 4 years with a clinical evaluation and a MCh using PtcO<sub>2</sub> as response performed every 6 months until age 4 and once per year thereafter. The clinical score improved in most children over time. Persistent wheezing was present in 31% of children after 2 years and in 20% of children after 4 years of follow-up. Children with persistent wheezing had a lower V<sub>max</sub>FRC at initial evaluation and a higher sRaw at the final follow-up visit as compared with asymptomatic children. BHR, as reflected by PD15%PtcO<sub>2</sub>, was greater in children with persistent wheezing as compared to asymptomatic children. The difference was apparent as early as 30 months, but a threshold value that reliably predicted subsequent progression could not be identified. The authors concluded that wheezing infants who develop asthma over a 4-year period have increased BR, although it was not possible to identify the threshold value that predicted future progression.

Studies using sRaw and CACH from age 2 years may provide the same kind of prospective data for prediction of ongoing asthma. Such important data are becoming available in a current study, the COPSAC study (4.1.4) (43), which will both provide data on Mch in infancy and CACH from age 4 and further on.

#### *Direct or indirect tests: pros and cons*

Direct airway challenges have been widely used and are well standardized. They are highly sensitive and can be used to exclude current asthma in a clinic population but are not specific to asthma (30). In fact MCh is more useful in excluding a diagnosis of asthma than in

establishing one because its negative predictive power is greater than its positive predictive power (115). MCh has been extensively studied in children and also with success in children from age 2 years. However, direct methods are probably not mimicking the pathophysiology of asthma to the same extent as indirect tests, and they are notoriously time consuming (121), using multiple step protocols for construction of dose response curves, which is a significant problem concerning testing among children in the age of 2 to 5. Furthermore, these tests also imply risks of systemic side effects such as flushing and headache and local effects such as dry mouth and cough. In addition, they are not at all very appealing and parents are probably more reluctant to allow their child to inhale a pharmacological agent than to let them undertake a non-pharmacological test.

Indirect challenge tests such as exercise, hyperventilation, hypertonic aerosols, as well as adenosine, may reflect more directly the ongoing airway inflammation and are therefore more specific to identify active asthma (30). In children, an exercise challenge is better than MCh at distinguishing asthma from chronic airway disorders such as CF, bronchiolitis obliterans, primary ciliary dyskinesia and bronchiectasis (146-148). Furthermore, natural stimuli, such as exercise and cold air inhalation, are more appealing and consequently we anticipate an increasing interest in the use of indirect airway challenges particularly in children. In fact, in a very recent publication from the ERS task force on indirect airway challenges the following working definition on an indirect challenge was: "Indirect challenges act by causing the release of endogenous mediators that cause the airway smooth muscle to contract, with or without effect in inducing microvascular leakage. Because the responses to these challenges are modified or even completely inhibited by ICS, the airway response to these challenges may be a closer reflection of active airway inflammation" (30).

#### *Studies on bronchial responsiveness in young children*

In a search of the literature for studies published since 1978 (~27 years) we found 34 studies which used bronchial challenge tests for various purposes in groups of asthmatic children, where all or some of the children qualified for the preschool age group. We have summarized the different studies beneath. The preferred method for assessment of BR in young children has, like in school children and adults (30), predominantly been direct airway challenges, since 23 studies preferably used methacholine, histamine or carbacholine (21, 22, 24, 34-42, 92, 97, 130, 131, 149-155). Only 11 studies used indirect challenge techniques (1-4, 7, 44, 47, 148, 156-158), of which half were performed in our laboratory (1-4, 7).

The major conclusions which can be drawn from the studies on direct methods are:

*PtcO<sub>2</sub>* has been the preferred method for assessment of response to MCh, since this method was involved in 16 of 23 studies either as primary outcome parameter (35, 36, 131, 149, 152, 153) or as reference method, when such methods as FOT (39, 40, 150), Rint (41, 92), sRaw (42, 130) or multiple combinations of different methods were evaluated (21, 22, 24, 40, 154). In all studies, except one (154), *PtcO<sub>2</sub>* was found reliable, very sensitive and useful for tracking changes to MCh (21, 22, 24, 39, 40, 92, 97, 150) and for separation between asthmatics and control subjects (35, 149) or between different phenotypes of asthma (131, 152, 153). This method was often at least as sensitive as comparators. However, *PtcO<sub>2</sub>* is a completely indirect method for assessment of BR, probably reflecting mismatch of lung perfusion and ventilation as a result of diffuse bronchoconstriction, without any reflection of airway mechanics and is therefore not suitable as measurement of baseline lung function, only BR. The method is however time consuming considering preparation time and the time needed to construct a response curve.

Studies employing various *auscultation* methods claimed to demonstrate a safe and sensitive method (37, 38, 151, 154), although one study found auscultation valueless and potentially dangerous (39). Still, this method obviously attracts attention by using good clinical,

medical skills, but inter-observer variation must be colossal and the method, like *PtcO<sub>2</sub>*, is only indirect and useless at baseline.

*IOS* or *FOT* showed good repeatability (24, 34), in some studies high sensitivity (21, 22, 40, 150, 155), but in one study *FOT* was deemed unreliable (39) for tracking changes to MCh which was supported by our results in later studies showing relatively poorer sensitivity of *IOS* as compared to sRaw (1, 5). In school children *FOT* (Rrs4) was as sensitive as spirometry in detecting change after bronchial allergen challenge (159) and *IOS* was more sensitive than *PEF* in detecting changes in *FEV<sub>1</sub>* (155).

*Rint* exhibited lowest sensitivity and poorest repeatability to MCh in comparisons between various methods (21, 22, 24) and was less sensitive to MCh compared to *PtcO<sub>2</sub>* (40, 92). Finally only 79% of children reached significant change in *Rint* when *PtcO<sub>2</sub>* had decreased 20% (41).

sRaw demonstrated good sensitivity (21, 22) and repeatability (24) in comparison to *Rint*, *IOS* and *PtcO<sub>2</sub>*. Challenge with carbacholine as measured with sRaw discriminated almost completely between asthmatics and healthy children judged by *BR* and *PD100* (42). *PD100* was significantly lower than in children with chronic cough but equal to *PD100* in children with wheezy bronchitis (42).

The effect of *ICS* on *BR* as judged with histamine challenge and sRaw was investigated in a *DBPC* study of disodium cromoglycate vs. beclomethasone dipropionate (*BDP*) for 2 months but was not reflected in any change of *BR*, whereas exacerbation rates improved in favor of *BDP* (130). Likewise, our own study (3) on effect of *ICS* did not show positive influence on *BR* as measured with MCh in contrast to *CACH*, attesting to reviews reporting *ICS* to reduce *BHR* to histamine or methacholine only to a small degree, an effect that shows both dose-response and time-response dependency (30, 160). See also 4.1.3.3

The use of MCh for diagnostic purposes in symptomatic young children was investigated in 2 studies using *PtcO<sub>2</sub>* (35, 149), and in a study using sRaw (42). However, none of these papers suggested any cut-off levels.

As previously mentioned, despite the holding that indirect tests are better at reflecting asthma pathophysiology (30), only a minority of published studies in young children employed indirect challenge methods. The major conclusions that can be drawn from studies applying indirect challenge testing are:

*Exercise testing* was used in the oldest study (158) and successfully separated between 3 to 5 year old asthmatics and healthy controls with high sensitivity of both *Rrs6* and *PEFR* measurements and also gave cut-off levels for each response. These young children ran along an indoor corridor accompanied by the investigator for 6 min and the pulse had to be at least 170 at the end of the test. Standardization aiming at constant sub-maximal workload was therefore obviously not possible, which will increase the risk of false negative tests, but obviously did not occur in this study. We have not been able to find any other publications using exercise testing, probably because others, like ourselves, found the test very difficult to apply and standardize in young children.

*Metabisulphite*, an inhaled indirect acting compound, was used for bronchial challenge in 36 3 to 20 year old patients (156) and response evaluated by *PD<sub>20%</sub>FEV<sub>1</sub>*. Responsiveness increased by age, but both response as measured by spirometry and the small number of patients in the preschool age group puts some limits to the value of conclusions in young children from this study. We did not find other studies using this compound in young children.

*Hypertonic saline* for inhalation was evaluated against MCh and responsiveness was measured with *FOT* (*Rrs<sub>6</sub>*) and *PtcO<sub>2</sub>* in 40 5 to 6 year old children (157), but none of the tests were sensitive for wheeze or clinical severity. This test appears to be very simple and inexpensive, but probably suffer the same disadvantage as MCh: the necessity to perform multiple steps and construction of dose response curve. No other reports using hypertonic saline have been found.



Adenosine was used for bronchial challenge and compared with MCh between 39 young children with asthma and 15 with various chronic airway diseases (148). The authors used the auscultation method and claimed to be able to separate between asthma and other chronic airway diseases, because MCh was nonspecifically positive in all patients, while adenosine primarily exhibited significantly higher responsiveness in asthmatics. Adenosine bronchial challenge, like MCh, is a multi-step procedure and needs construction of response curves. In this study endpoint was not reached in 8/15 children. Furthermore, the auscultation method is interesting, but does not provide a measurement of airway mechanics, as previously discussed.

CACH was performed in a great number of 6 year old children taking part in the Tucson Children's Respiratory Study, a longitudinal study of respiratory illness involving children enrolled at birth between May 1980 and October 1984 (44). The purposes were to assess BHR at age 6 years in a group of children who did not have a diagnosis of asthma and who did not have a history of respiratory symptoms compatible with such a diagnosis and to study the relation between BHR and the incidence of new cases of asthma or asthma-like symptoms during the subsequent years. BHR at age 6 was associated with subsequent development of a diagnosis of asthma but this effect was not independent of atopy and mild wheezing at age 6. The study hardly meets the criteria for inclusion in this review both because of age (6 plus, <7) and the method by which responsiveness was measured,  $V_{max}FRC$ , but it points to

areas of research that ought to be repeated in young children of 2 to 5 years using e.g. CACH and sRaw (see 7.).

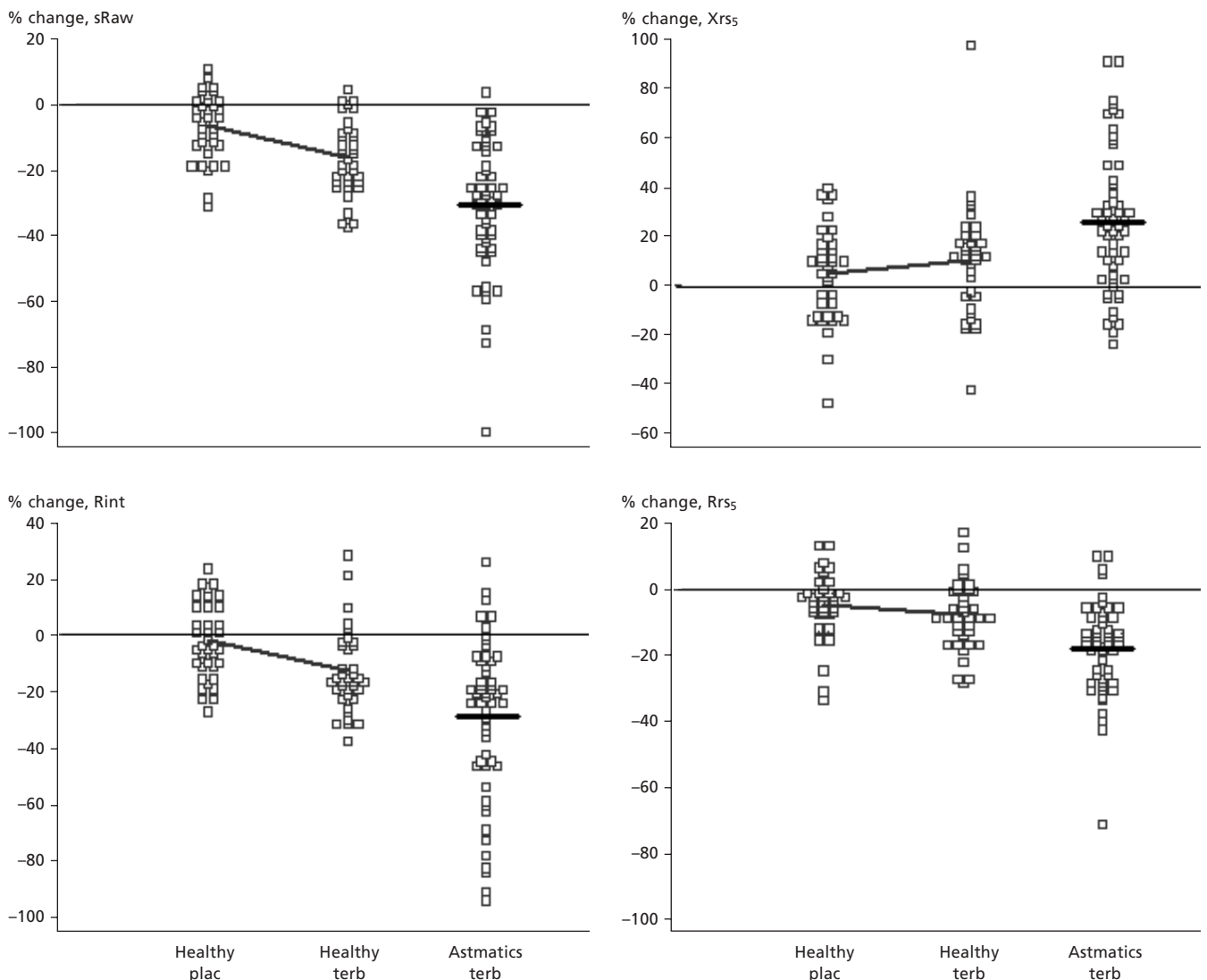
DACH and sRaw was used in a recent study in 5 year old children demonstrating that persistent wheeze in contrast to other wheeze phenotypes was associated with increased BR (47).

The usefulness of CACH and sRaw as indicators of efficacy of various anti-asthmatic medications, including ICS is covered in 4.1.3.

In conclusion, BR is readily assessable in young children, whether healthy or suffering from asthma or other chronic airway diseases. The candidate challenge procedures and response measurements are multiple and have been reviewed above. A clinical test of BR in young children should be simple, easy to apply and of short duration, and have documented capability to function as a diagnostic tool, evaluate disease severity and efficacy of anti-asthmatic treatment, specifically ICS, and finally serve as a prognostic factor for ongoing asthma. We believe to have shown and documented by clinical trials, that the best candidate challenge test to meet most of these criteria is the CACH using sRaw as response measurement. The literature did not provide evidence for usefulness of MCh in young children.

#### Limitations to our studies

Retrospectively, the study design in the "feasibility of CACH study" (I) (1) probably did not allow for a balanced comparison of sRaw, Rint and IOS, giving all methods even credits. In event of a future comparison this should be done with each method on separate oc-



**Figure 4.1.3.1.a.** Bronchodilator response in healthy and asthmatic subjects and response to placebo in healthy subjects expressed as percent of predicted values (%pred) for sRaw, Rint, Rrs<sub>5</sub>, and Xrs<sub>5</sub>. Means are indicated by connecting lines (healthy subjects) and horizontal bars (asthmatic subjects).

casions one day apart and exactly at the same time point after challenge in order to give full credit to all methods. Alternatively 3 challenges, one for each method, at least 2 hours apart (refractoriness) could be done. However, both of these solutions detract from acceptance of child and especially parents. Our approach was a compromise in this respect.

### 4.1.3 Efficacy of asthma medication

#### 4.1.3.1 Bronchodilators

##### Discriminative capacity of bronchodilator responsiveness

In 55 children with asthma and 37 healthy children (aged 2 to 5 years), we assessed the ability of sRaw, Rint and IOS to discriminate between the bronchodilator responsiveness (BDR) in the patients and control subjects (5). Measurements were made before and 20 min after inhaling terbutaline from a metered-dose inhaler and spacer. All measurements detected a greater bronchodilator response in the patients as compared with the control subjects attesting to the increased bronchomotor tone in asthmatics, **Figure 4.1.3.1.a**. However, healthy young children have an inherent bronchomotor tone as seen from a 16%, 13%, 11% and 8% change in sRaw, Rint, Xrs<sub>5</sub> and Rrs<sub>5</sub>, respectively, after inhaled  $\beta_2$ -agonist, **Figure 4.1.3.1.a**. Using ROC curve analysis we found sRaw to have a sensitivity of 66% and a specificity of 81%. Rint had a sensitivity of 58% and a specificity of 70%. Rrs<sub>5</sub> had a sensitivity of 76% and a specificity of 65% and Xrs<sub>5</sub> had a sensitivity of 33% and a specificity of 89%. We concluded that a 25% decrease in sRaw in response to a bronchodilator provides the best discrimination between asthma and health in young children.

##### Bronchoprotective effect of bronchodilators

In 12 children with asthma, aged 2 to 5 years, we studied and compared the bronchodilatory and bronchoprotective efficacy of formoterol, a long-acting  $\beta_2$ -agonist, and salbutamol delivered from a dry-powder inhaler and used with a spacer (4). Using a double-blind crossover design, formoterol and salbutamol produced equivalent decreases in sRaw at 3 min. The bronchodilator effect of formoterol was sustained for at least 8 hours and began as rapid as salbutamol as shown by measurement at 3 min post-dose, whereas the effect of salbutamol lasted less than 4 hours, **Figure 4.1.3.1.b**. At a screening visit, hyperventilation of cold air produced a 50% increase in sRaw. Pretreatment with formoterol provided ~80% protection for at least 8 hours and was consistent from 15 minutes post-dose until 8 hours later, whereas salbutamol provided less than 4 hours of bronchoprotection. The initial time course of protection

by formoterol and salbutamol was similar as suggested from the equal protection at 15 minutes post-dose, **Figure 4.1.3.1.b**. We concluded that administering formoterol as a dry powder inhaler with a spacer to 2- to 5-year-old children with asthma provided rapid and sustained bronchodilation and bronchoprotection for at least 8 hours.

We were first to provide data to suggest that the initial time course of protection from formoterol and a SABA was similar. This similarity was later confirmed in an adult study using EIB as outcome (161).

#### Bronchodilators in treatment, diagnosis, prognosis and monitoring of asthma

The pharmacologic effects of SABA and LABA are well known as well as their clinical effects from multiple studies primarily in asthmatic adults and school children. Their role in asthma management is well established and described in asthma guidelines (32).

##### Treatment

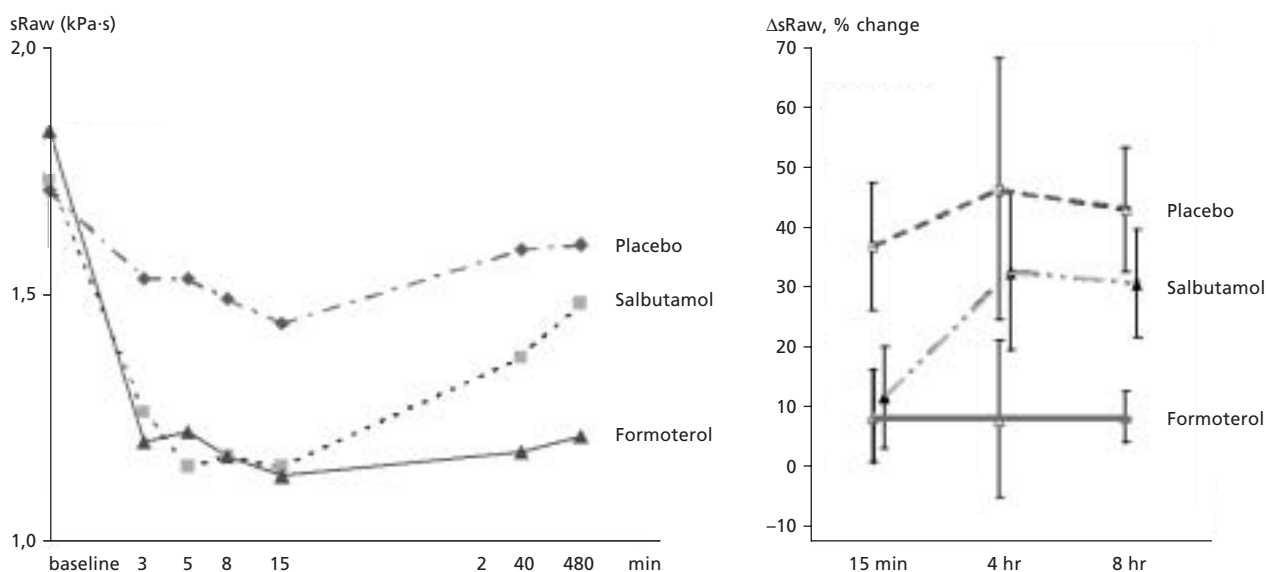
Bronchodilators are primarily used as reliever medications during exacerbations in asthma and for acute bronchospasm in various conditions and secondly for protection against e.g. exercise induced asthma (32). The successful introduction of LABA and ICS in fixed combination prescribed for regular daily therapy emphasizes their protective role against exacerbations (162, 163). Bronchodilators are also widely used in other chronic lung diseases mainly for their bronchodilatory effect and positive influence on pulmonary mucociliary clearance (164).

##### Diagnostic, prognostic & monitoring purposes

Their role in the diagnosis of asthma is well-recognized (165-167). Typically, a  $\geq 12\%$  to 15% increase in FEV<sub>1</sub> 15 to 20 min after a rapid acting  $\beta_2$ -agonist is indicative of asthma in both children and adults (94, 165, 168), and a large BDR at baseline predict improvement in FEV<sub>1</sub> during long-term ICS treatment (169). Reversibility testing is commonly used for assessment of asthma, since a positive reversibility test in a patient on controller therapy is considered indicative of poorly controlled asthma.

#### Studies on Bronchodilator Responsiveness in Young Children

BDR is easy to recognize clinically (at a distance and by stethoscope) in a moderate to severe asthmatic young child, but completely impossible in less severe asthmatics or in asymptomatic or healthy young children. We found 20 papers reporting on BDR in young or



**Figure 4.1.3.1.b.** Left: Mean sRaw curves in absolute values pre-dose (baseline) and through 8 h after administration of placebo, salbutamol, and formoterol single dose from a mechanically actuated DPI using a spacer. Right: Maximal percent increase in sRaw (expressed as percentage of baseline) after CACH at 15 min, 4 h, and 8 h after single dose of placebo, salbutamol and formoterol. Mean and 95% CI are shown.

preschool children measured by different lung function methods from the last 27 years, including 4 studies from our laboratory (4-6, 22). The majority of studies used the short-acting  $\beta_2$ -agonist (SABA) salbutamol in a dose range between 200 to 800  $\mu\text{g}$  or equivalent doses of another SABA, terbutaline, or formoterol or salmeterol, both long acting  $\beta_2$ -agonists (LABA). By using such doses, which are at the top of the dose response curve, it is anticipated that all individuals, healthy or not, exhibit more or less BDR. However, very little is known about the best method to use for detection and measurement of the magnitude of such BDR in young children with asthma or other chronic lung diseases or in healthy children. Most of the studies were designed to evaluate different lung function techniques in the assessment of BDR in various situations. Interestingly, no reports were found on the previously mentioned auscultative method, attesting to the limitation of this method, likely because of its high dependence on sound phenomena that may not be present despite significant flow limitation.

#### *BDR in healthy young children*

BDR was assessed exclusively in healthy children in two studies (84, 89). Mean inspiratory and expiratory Rint decreased significantly ( $-15\%$  and  $-12\%$  of predicted values, respectively) in 91 2 to 7 year old children (84), confirming our observation of  $-13\%$  decrease (of predicted value) in inspiratory Rint in 2 to 5 year old healthy children (5). Another study using IOS in 108 2 to 7 year old children showed significant mean change in  $R_{rs5}$  of  $-0.187 \text{ kPa}\cdot\text{s}\cdot\text{L}^{-1}$  or  $-19.2\%$  from baseline, and estimated a lower reference limit of  $-36.9\%$  (89). Both of the presented values of BDR are approximately double of the corresponding values in our study and the cut-off level is very close to the lower 95% limit in our *asthmatic* patients (5), but close to the estimated cut-off level in another study (88). This difference is not readily explained by difference in use of facemask as argued for the observed difference in mean values in healthy (3.2.6). Difference in passive smoking (not allowed in our reference materials (5, 25) could explain a lower BDR in our study, but not the observation of a significant lower baseline in the Finnish study. Such differences may disqualify this method and obviously calls for further standardization and studies on inter-center repeatability of this method.

#### *BDR used in order to discriminate between asthmatics and healthy young children*

In a study using IOS, neither baseline  $X_{rs5}$  or  $R_{rs5}$ , or BDR were significantly different between healthy and asthmatics (88), which is in contrast to our study (5) and a Finnish study (138), but may be explained by selection bias. A change in  $R_{rs5}$  of  $>40\%$  was proposed as "positive" BDR (88), which is much higher than our finding as discussed above. NO provided better discriminative capacity than BDR as measured by change in  $R_{rs5}$  as percent of predicted (138). The discriminative capacity of BDR as measured with IOS was slightly better than our findings. Marotta and colleagues (72) found that BDR measured with IOS ( $R_{rs5}$  &  $R_{rs10}$ ) separated asthmatics and non-asthmatics and moreover that  $R_{rs5}$ ,  $R_{rs10}$  &  $X_{rs10}$  separated atopic asthmatics and non-atopic asthmatics. Beydon and colleagues using expiratory Rint (65) reported significant greater BDR in asthmatics than in controls,  $-23\%$  versus  $-13\%$  of predicted, respectively, and proposed that a 35% decrease had a likelihood ratio of 3 to separate asthmatics from healthy controls. These findings are in concordance with our results (5). Wheezers exhibited larger BDR as opposed to recurrent or persistent coughers and healthy as measured by Rint (63) and a larger proportion of wheezy children exhibited significant BDR as compared to coughers and healthy (86). We did not find other studies on BDR using sRaw to discriminate between healthy and asthmatic young children.

#### *Assessment of BDR in a group of asthmatic young children*

Some studies did not have control subjects as reference. In one of

these studies BDR was measured with Rint in children with a history of wheezing (76). BDR of  $>2\text{SDw}$  was shown in 82% without active wheeze and in 88% of actively wheezing children, disclosing ability of Rint to reflect the changes, but also emphasizes the need for control subjects and blinding of the observer to reduce bias in such studies as discussed under Future Directions. Similar to our findings of BDR in asthmatics, another study demonstrated a mean change of  $-24\%$  in Rint corresponding to 11% increase in  $\text{FEV}_1$  (170), and Arets and colleagues (95) reported feasibility of Rint in a large sample of children from 0.8 to 16 years, but only reported on BDR in 35, primarily school children, exhibiting a mean change of  $-22.5\%$ , close to our and other's results with Rint.

In addition to our study (4) we found one other report on BDR using LABA in young children. In a single-blind placebo controlled study with the LABA salmeterol and the SABA albuterol BDR was measured with IOS ( $X_{rs5}$ , Jaeger) (171). This study claimed to show significant BDR compared to placebo from 30 min to 540 min, but only when salmeterol was administered with metered dose inhaler (MDI), while this effect was present only at 540 min post-dosing when administered with Diskus. However careful review of the results suggests that the authors did not interpret their data correctly, since both salmeterol MDI and Diskus offers significant and persistent change in  $X_{rs5}$  judged from their figure of result (171). BDR to albuterol exceeded salmeterol (MDI and Diskus) within the first hour, which is difficult to explain and seems to raise doubt to methodology and power of this study.

#### *Assessment of BDR after MCh or in acute asthma*

Bronchoconstriction after MCh provides an imitation of acute asthma and is an ideal model for testing of BDR. This was used in young children after EIB and showed that  $R_{rs6}$  and PEFr normalized in all 23 participants (158). Klug and colleagues (22) compared different methods in the assessment of BDR after MCh and found that the order of sensitivity by methods was  $s\text{Raw} > X_{rs5} > \text{Rint} > \text{PtcO}_2 > R_{rs5}$  for detecting BDR, somewhat different from the order of sensitivity to detect the foregoing change after MCh and from the order of sensitivity when testing BDR in children with acute asthma:  $X_{rs5} > s\text{Raw} > \text{Rint} > R_{rs5} > \text{PtcO}_2$  (22). Rint also changed significantly in 11 of 12 asthmatic children relative to both post MCh values and baseline (92). Dubus et al (172) assessed the influence of spacers with different electrostatic charge on BDR in 64 3 to 6 year old children and found a decrease of 152% in sRaw corresponding to 20% increase in  $\text{FEV}_1$  after maximal MCh, however without showing any difference between various spacers.

#### *BDR in cystic fibrosis*

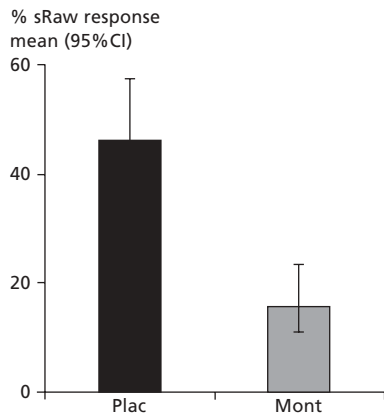
This is covered in 4.2.3

#### *Bronchodilators in the protection against asthma*

Except for our own study on salbutamol and formoterol referenced above (4), we did not find other studies in young children having tested this important pharmacological property of bronchodilators. Young asthmatic children are particularly in need of bronchodilation and bronchoprotection of long duration because intermittent treatment with SABA is often insufficient as the treatment decision and drug delivery are dependent on a trained caretaker, in most cases the mother, who often has to hand over the observation and care of the child to others for large parts of the day. LABA "as needed" would be of significant clinical benefit for young asthmatic children. During symptomatic periods, the parents could give the child one treatment in the morning to ensure effective symptomatic relief throughout the day.

#### *Conclusions from studies on bronchodilators are:*

Healthy young children exhibit significant BDR as measured with sRaw (4, 5), Rint (5, 84) and IOS (5, 89). The power of BDR as measured with IOS to discriminate between health and asthma



**Figure 4.1.3.2.** Percent change from baseline in specific airway resistance (%sRaw) in young asthmatic children after cold, dry air hyperventilation. The bronchoprotective effect of the leukotriene receptor antagonist montelukast (MONT) was significantly different from that of placebo (PLAC). Mean and 95%CI is shown.

seems controversial. We found  $Xrs_5$  useless, but  $Rrs_5$  of some value (5), while others found IOS without any power (88), in contrast to 2 studies showing good discrimination (72, 138). Rint exhibited reasonable discriminative capacity in one study (65) but we showed that this capacity was inferior to both  $Rrs_5$  and sRaw (5). Very few studies have documented and quantified BDR in young children with acute asthma (22) and the important aspect of bronchoprotection from both SABA and LABA was reported in one study only (4).

#### 4.1.3.2 Leukotriene Receptor Antagonists (LTRA)

To determine whether a leukotriene receptor antagonist (LTRA) provides bronchoprotection in preschool asthmatic children, we did a DBPC crossover study in 13 asthmatic children aged 3 to 5 years (2). Hyperventilation of cold, dry air produced a 46% increase in sRaw when the children took placebo, and a 17% increase after 2 days of the LTRA montelukast (5 mg per day), **Figure 4.1.3.2**. The benefit was seen irrespective of whether the children received ICS. We concluded that LTRAs may be of value in treating preschool asthmatic children.

#### Leukotrienes and airway disease

Leukotrienes (LTs) are predominantly leukocyte-generated and leukocyte-released lipid mediators that act as direct bronchoconstrictors and promote airway inflammation in asthma and virus-induced wheezing by mediation of vascular leakage, inflammatory cell recruitment, and possibly airway remodeling (173).

CACH is hypothesized to stimulate the release of inflammatory mediators (3.3.1.1). Suggestive evidence indicates that LTs may be involved in the responsiveness to CACH and exercise, since inhibition of a precursor enzyme 5-lipoxygenase, was associated with an increased tolerance to cold, dry air (174) and blunted the obstructive response to exercise during inhalation with frigid air (175) in adult asthmatic individuals.

Corticosteroids generally reduce the *in vitro* production and release of LTs, while for unknown reasons such effect does not seem to convincingly appear *in vivo* (173). This observation is probably to a certain extent the explanation for the ongoing complaints of symptoms and objectively detected signs of e.g. exercise induced asthma in asthmatic patients despite treatment with conventional doses of ICS (173). Likewise our studies showed significant BHR to CACH and/or DACH in a number of ICS treated young children (1-4, 7) attesting to the limitations of ICS as complete controllers.

#### The role of leukotriene receptor antagonists

Recognition of the importance of LTs in the pathogenesis of asthma has led to the development of LTs modifiers, the first new class of drugs for the treatment of asthma to become available in nearly 3

decades. They exert anti-inflammatory effects that attenuate cellular infiltration and BHR and complement the anti-inflammatory properties of ICS. Clinical trials with the LTRA montelukast demonstrated efficacy in improving pulmonary function and the control of asthma exacerbations in adults (176) and school children (177) with persistent asthma. Moreover, exacerbations were significantly reduced in preschool children (178). In addition, montelukast significantly reduced symptoms and exacerbations from respiratory syncytial virus post-bronchiolitis in non-asthmatic infants (179). Furthermore, a recent study demonstrated efficacy of montelukast in the reduction of viral-induced asthma exacerbations in children aged 2 to 5 years with a history of intermittent asthma symptoms (180). Compared to placebo, exacerbation rates were reduced with 31.9%, the rate of ICS courses was reduced, and the median time to first exacerbation was delayed by approximately 2 months. LTRAs also ameliorate BR to exercise in school children (181) and adults (182) by limiting the decrease in and accelerating the recovery of FEV<sub>1</sub> without the tolerance issues (183, 184) and concerns about increased exacerbation rates seen with daily LABA use (185, 186). However, LTRA do not apparently reduce the response to MCh (182), probably reflecting that LTs do not act in the response to this direct challenge. In this respect our study in young children (2) attests to the efficacy of LTRA in the treatment of young children with CACH induced bronchoconstriction, whether treated with ICS, and point to a role of LTs in this type of BHR. We tested the effect 12 hours after the last of 2 doses given 24 hours apart in the evening (2), but in contrast to prevalent practice, a single dose of montelukast may be all that is required to produce a beneficial effect after 12 hour (175, 187) and there is no need to establish a stable blood level, hence long-term use is not a prerequisite.

#### 4.1.3.3 Inhaled corticosteroids

The efficacy of ICS in young children has already been documented by improvement in symptom score and decrease in use of rescue treatment with SABA. However, we provided the first report of the efficacy of ICS in asthmatic children aged 2 to 5 yr to be based on objective measurements of lung function and BR (3). The study was explorative, evaluating objective measures together with conventional measures of symptoms as primary outcomes of asthma disease activity. In 38 asthmatic children aged 2 to 5 years, we assessed whether objective measures of lung function including BR to CACH could serve as supplemental tools in assessing response to therapy. In a randomized, DBPC trial, ICS (budesonide 400 µg twice daily) produced a decrease in nighttime and daytime symptoms, increased number of symptom free days, reduced exacerbation rates and decreased daytime, but not nighttime, use of rescue medications. Budesonide also improved measurements of resistance and BR to CACH, **Figure 4.1.3.3**, (but not methacholine responsiveness). We concluded that ICS decrease symptom scores and improve objective measures of lung function including BHR in preschool children.

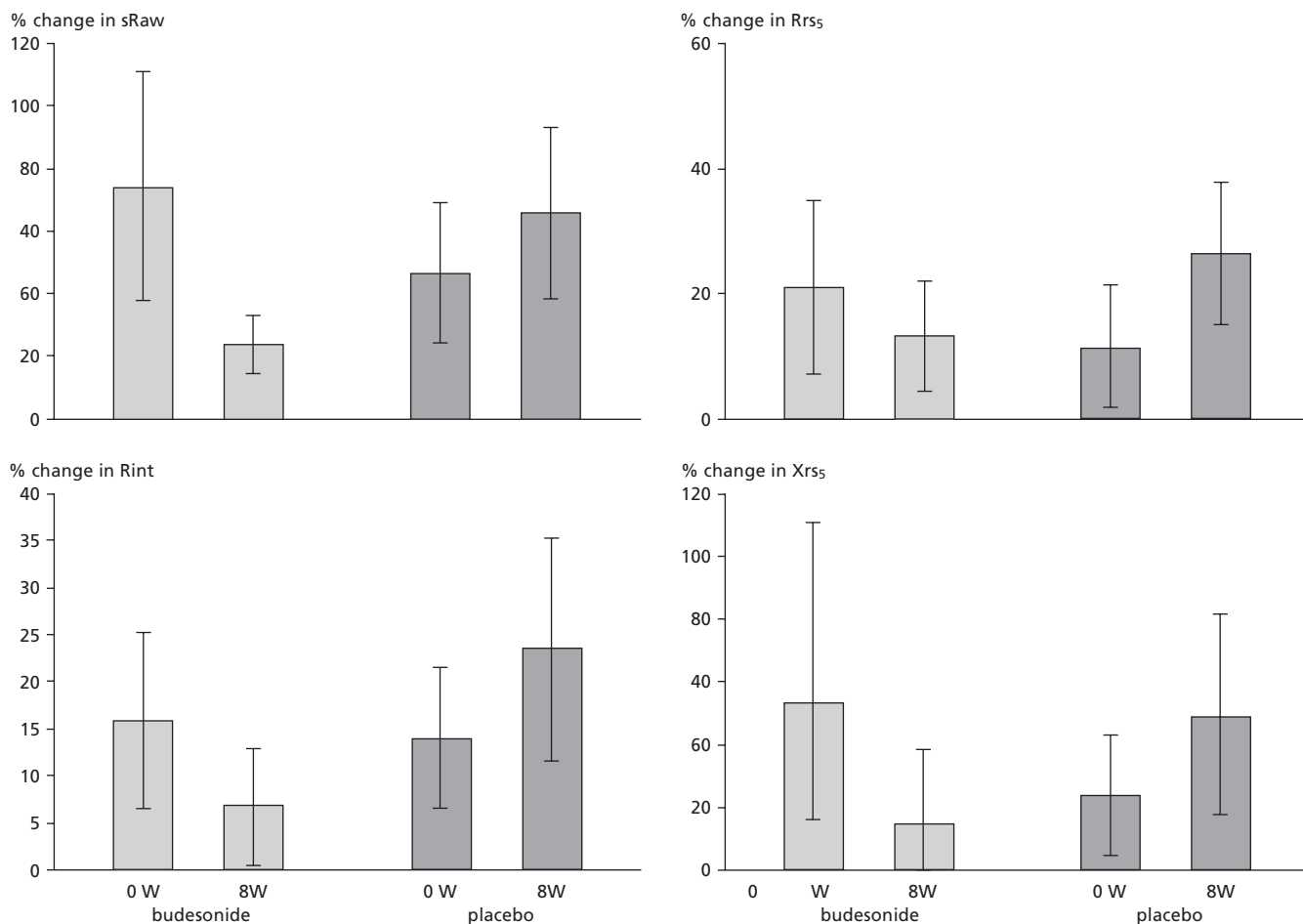
#### Efficacy of ICS in young children

The efficacy of ICS in preschool children has been primarily documented in studies monitoring health outcomes such as symptom score, use of rescue treatment, and quality of life score. Methods such as lung function measurements and monitoring of inflammatory markers have been used in a limited number of studies.

#### Symptoms, rescue and quality of life

The very first published study to show efficacy of ICS in young children was a DBPC trial by Bisgaard and colleagues (188). Since then at least 10 other DBPC studies (3, 189-197) and two double blind controlled trials (130, 198) have been published on this issue and all showed significant and clinically relevant improvements in young asthmatic children treated with ICS.

The dose of budesonide in our study was 400 µg twice daily (3), which is on the flat part of the dose-response curve and above the



**Figure 4.1.3.3.** Change (%) from baseline in measures of lung function (sRaw, Rint, Xrs<sub>5</sub>, and RRs<sub>5</sub>) in response to cold air challenge at randomization (0 W) and after 8 weeks (8 W) of treatment with budesonide or placebo. Mean (95% CI).

recommended dose for young children with moderate to severe persistent asthma. Due to the explorative nature of this study we deliberately chose the high dose to increase the likelihood of detecting any possible effect on lung function and BHR, since treatment effect was highly expected. Importantly, despite the high dosage of budesonide, it was not possible to eliminate asthma symptoms in all of the children in the study. Generally, the study groups of young asthmatic children do not become symptom-free. ICS typically reduce the number of asthma exacerbations by half as seen in our study where we found a total of 12 exacerbations in the active treated group and 29 in the placebo group, giving exacerbation rates of 3.7/yr versus 9.3/yr for budesonide and placebo, respectively, supported by similar observations in other studies (192, 197). However, ICS do not prevent exacerbations completely leaving room for additional therapy, e.g. LTRAs, improved therapy or alternative dosing schedules.

The design of the present study was biased by regression toward the mean, since randomization took place immediately after a period of significant symptom scoring. This was reflected by the pronounced placebo effect, which is seen in most trials on asthma in young children, and the study results should therefore be interpreted in the light of these unfavorable odds, strengthening the conclusions of the study.

#### Lung function

A very limited number of studies have reported on lung function as primary endpoint in trials of ICS in asthmatic children under age 6. Lung function and BHR were secondary endpoints in our study. We found that Rint and IOS measurements of baseline resistance and reactance mirrored the previously mentioned clinical effect, whereas sRaw seemed less sensitive in detecting long-term changes in baseline lung function (3).

Two studies were conducted predominantly in infants. Maayan and colleagues (199) reported improvement from ICS of the airway conductance measured in whole-body plethysmography in 9 sedated asthmatic infants. Another study showed improvement from beclomethasone dipropionate (BDP) in combination with salbutamol of TGV and airway conductance in a 6 weeks study of 29 infants aged 2-25 months with recurrent wheezing (200). In a DBPC trial in 19 young asthmatic children the effect of a daily dose of 400 µg budesonide, pMDI via a spacer for six weeks was suggested to reduce hyperinflation reflected in the functional residual capacity measured by a helium dilution method (201).

Rint was used as outcome parameter in 61 2 to 5 year old children with intermittent wheeze completing a 6-week randomized controlled crossover trial of fluticasone propionate (100 µg, twice daily), followed by a 10-week parallel extension in 44 children (202). Rint showed significant improvement in sensitized children in contrast to non-sensitized and deteriorated after stopping treatment, suggesting relevance of treatment with ICS in sensitized children with intermittent wheeze.

#### BHR

As mentioned above we reported the efficacy of ICS (budesonide 800 µg) for 8 weeks on improvement of BHR as measured by CACH method, but not by MCh. The beneficial effect of ICS on BHR to CACH was reflected in sRaw as well as Rint and IOS measurements (3). It has been demonstrated repeatedly that, despite significantly improving symptoms and decreasing airway inflammation, ICS produce, at best, a modest decrease in BHR as measured by histamine or MCh. This observation has been made in adults (203) as well as children with asthma (204).

ICS did not improve BHR in a previously referenced double-blind cross-over trial (130). Treatment for 2 months with nebulized BDP

300 µg daily and disodium cromoglycate was compared in 15 children 3-5 years of age with a history of recurrent wheezing. BR to histamine was measured by sRaw and although an improvement in symptoms was reported, no significant change was seen in BR.

The rapid thoracoabdominal compression technique was used to measure flow at functional residual capacity, and for assessment of BHR to histamine in 38 infants aged 5-18 months with a history of 3 episodes of wheezing or persistent wheezing for more than 4 weeks. Only BHR improved significantly from 400 µg of BDP pMDI via a spacer with face-mask in contrast to no change in symptoms or lung function in this parallel DBPC study (205).

#### Inflammatory markers in exhaled air

A significant rise in exhaled NO subsequent to dose reduction of ICS in young asthmatic children of 2 to 5 years attested to the inflammatory nature of the disease and the anti-inflammatory control by ICS in young asthmatics (206). No other studies on ICS in young children have reported on inflammatory markers.

### 4.1.4 Lung function as outcome in early intervention studies

#### Whole-body plethysmography

Baseline lung function, bronchodilator and CACH responsiveness measured with sRaw has been chosen in combination with many other outcome parameters in The Copenhagen Prospective Study on Asthma in Childhood (COPSAC) investigating the relationships among genetic, environmental, and lifestyle factors in the development of atopic diseases in 411 high-risk children with the aim of developing evidence-based prevention strategies (43). sRaw is measured yearly from the age of 2 years (21), BDR is measured at 3 and 5

years of age and CACH responsiveness is measured at the age of 4 and 6 years. Data from this study are emerging.

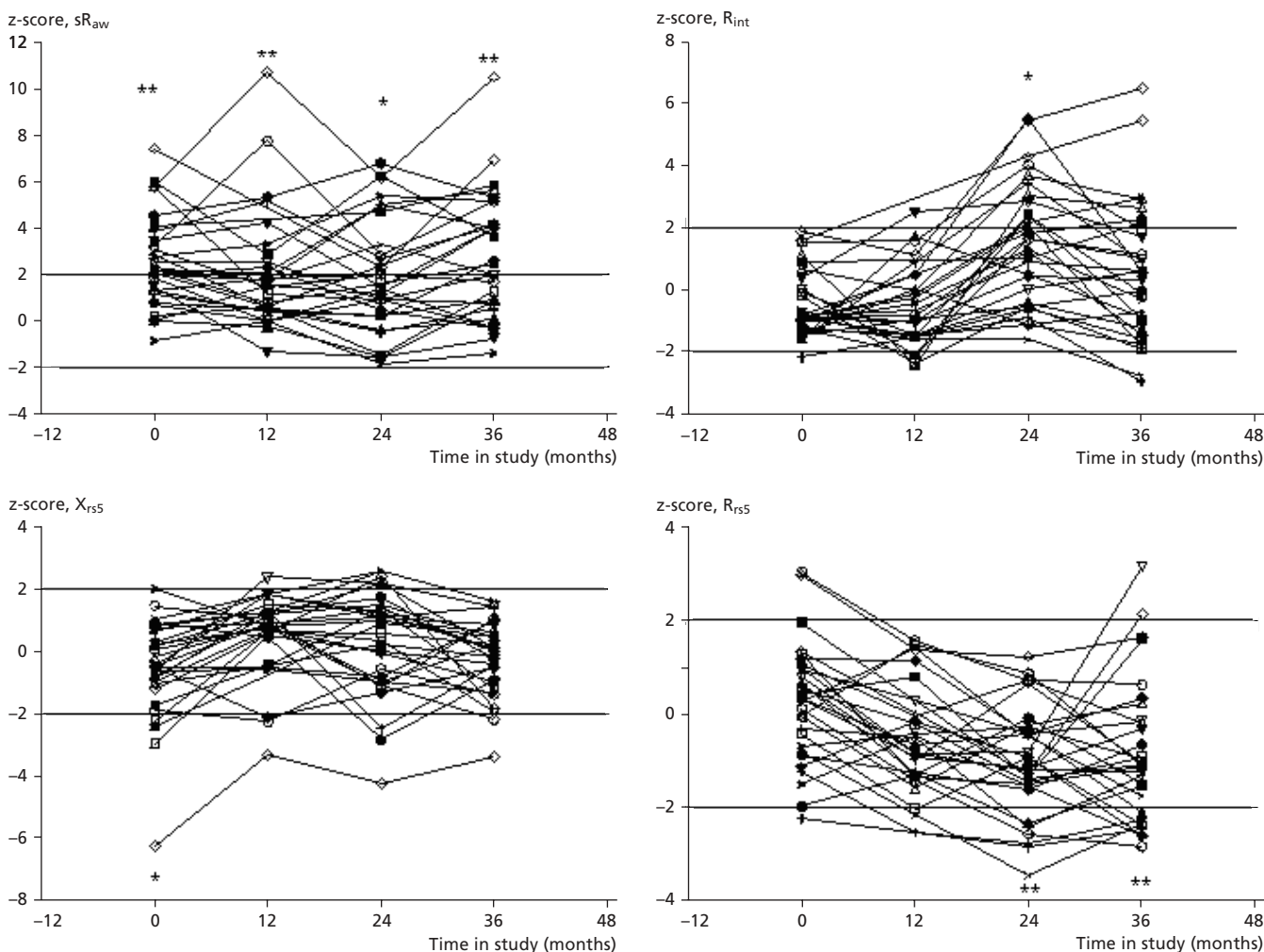
In another early intervention and cohort study investigating whether environmental control during pregnancy and early life affects sensitization and lung function at the age of 3 years, 251 high-risk children were prenatally randomized to stringent environmental control (active) or no intervention (control) (207). sRaw was measured at the age of 3 years and was significantly better in the active group despite increased risk of mite sensitization. As part of the same study, based on parentally-reported history of wheeze and sRaw at age 3 and 5 years, the children were classified as never wheezers, transient early wheezers, late-onset wheezers or persistent wheezers (47). sRaw was higher in the latter compared to the other groups. In children who had wheezed by age 3 years, the risk of persistent wheeze increased with increased sRaw (odds ratio, 95% CI: 5.2, 1.3-22.0). Increasing sRaw and sensitization were significant, independent predictors of persistent wheezing.

#### Interrupter technique

In a Dutch study, Rint was compared between different wheezing phenotypes in 4-year-old children. All children participated in the Prevention and Incidence of Asthma and Mite Allergy cohort, a prospective birth cohort of more than 4,000 children (135). At 4 years of age, data on Rint plus wheezing phenotype were available for 838 children. Persistent wheezers had significantly higher Rint values than children with early wheeze and those who never wheezed independent of the atopic status of the mother.

#### Impulse oscillometry

To our knowledge there are no published studies on IOS data in co-



**Figure 4.2.1.** Individual measurements in children with cystic fibrosis expressed as z-scores for each method (sRaw, Rint, Rrs5, Xrs5) and at each of the time points in the study are shown. \*) p < 0.05; \*\*) p < 0.001.

hort or early intervention studies as yet. However, The Childhood Asthma Research and Education Network (CARE) in USA designed a randomized clinical trial to determine if persistent asthma could be prevented in children at a high risk to develop the disease (73). They chose the IOS method presented by Klug & Bisgaard (22) as one among many other secondary outcome parameters.

#### 4.2 CYSTIC FIBROSIS

In 30 children with CF, aged 2 to 8 years, we measured sRaw, Rint and IOS on 5 occasions during a 4-year prospective study, including measurement of BDR on two different occasions and to cold air on one occasion. Spirometry was assessed from school age (6). To allow for longitudinal tracking of lung function, both within each method and from e.g. sRaw to FEV<sub>1</sub> and for comparison of methods, all data were transformed and presented as z-scores.

##### 4.2.1 Discrimination between health & disease

Mean sRaw was consistently and statistically significantly abnormal, exhibiting a mean z-score (SD) of 2.52 (2.02) at the start and was 2.74 (2.02) 36 months later as shown in Figure 4.2.1.

The proportion of patients exhibiting abnormal (high) z-score of sRaw at one or more visits ranged from 37% to 57%. Approximately 66% of the patients did not change their "track" during the observation period, which was also confirmed by Spearman test showing that the order rank at end of study for all tests did not differ much from the order rank at inclusion. However, neither Rint nor IOS demonstrated consistent abnormal levels, leaving sRaw as the most promising parameter for clinical use in young children with CF. In support of our findings a recent publication on a cross sectional study reported significantly higher sRaw in young children with CF than in control children (57).

Rint was significantly increased in young children with CF as compared with healthy control subjects, but despite this fact only 5 of 40 patients had Rint values outside normal range (64), which lends support to our findings with Rint as a rather insensitive measure in CF, probably explained by an unachievable instantaneous pressure equilibration in a multi-compartment CF lung during the limited equilibration time of only 80 to 100 ms usually employed. In fact, it has been found that this insensitivity of Rint increases with severity of the obstruction (208). Equilibration time should probably be longer, but this needs to be investigated.

The finding of a consistent abnormal sRaw was further confirmed by spirometric values showing abnormal FEV<sub>1</sub> with mean z score (SD) of -1.2 (1.2) from the first satisfactory measurement, at a mean age (range) of 6.1 (4.9-7.5) years until the last measurement, 4 years from inclusion, showing further reduction to -1.85 (1.2). At this last visit the mean age (range) was 9.9 (6.8-12) years.

##### 4.2.2 Bronchial challenge and responsiveness

We also studied responsiveness to CACH and although patients with CF as a group demonstrated statistically significant change (11.8%) from baseline in response to CACH as measured with sRaw, the responsiveness did not differ significantly from the responsiveness in healthy subjects (6). However, we found 7 individuals with a positive test as defined by an increase in sRaw >20% from baseline (4.1.2).

Studies using MCh have demonstrated that between 21% and 56% of subjects with CF exhibit BHR (209, 210). However, BHR as demonstrated with indirect testing is probably less frequent since this test is more specific in distinguishing "asthma-inflammation" from other types of inflammation as also shown by using adenosine 5'-monophosphate (AMP), another indirect challenge test, in children (147, 148).

The demonstration of BHR in CF patients has no importance for diagnosis, but has a place in helping the clinician to understand the rationale of using anti-asthma medication in CF individuals. The rationale for general use of ICS in CF is still not resolved, although we have previously contributed in this area of research (211).

##### 4.2.3 Response to Bronchodilators

Our study demonstrated that patients with CF as a group did not differ from healthy subjects in BDR, although statistically significant BDR within group was demonstrated in all parameters (sRaw, Rint and IOS) at 2 different visits 1 month apart (6). Furthermore, mean sRaw remained abnormal at both visits, with a postbronchodilator z score of 0.98 (95% confidence interval, 0.38 to 1.57) and 0.90 (95% confidence interval, 0.29 to 1.50), respectively. A positive test defined as decrease in sRaw >25% (4.1.3.1) was found in 30% and 21% at the 2 visits respectively, as measured with sRaw. No other studies have reported on BDR in CF measured with sRaw.

None or a very limited number of positive tests were seen with Rint (6), which is in accordance with previous findings on expiratory Rint showing no significant difference in effect of salbutamol between children with CF and control subjects (64). No other studies have been investigating BDR with Rint in CF.

Because forced expiration may cause paradoxical effect on unstable CF airways during FEV<sub>1</sub> measurement, FOT was suggested as an additional measure to spirometry in the evaluation of BDR in school children with CF (212). In 13 of 20 schoolchildren, the decrease of Rrs<sub>6</sub> was >12%, but no control subjects were measured for comparison. However, in a parallel report on IOS in young children the same group argued for a change of 41% in Rrs<sub>5</sub> as cut-off (88), leaving the change of 12% far inside the normal range of BDR. Moreover they found Rrs<sub>6</sub> useless for baseline measurements with poor correlation to FEV<sub>1</sub>. The liberal use of  $\beta_2$ -agonists in CF patients (212) has no foundation in real data on lung function measurements (6). The evidence to support this liberal use in young children with CF is needed.

Conclusion: sRaw may be a useful tool in CF during early childhood. Reduced lung function was documented from consistently abnormal levels of sRaw and FEV<sub>1</sub> during the study. Bronchodilator responsiveness and response to cold air challenge were normal.

## 5. CONCLUDING REMARKS

Whole-body plethysmography (sRaw) was superior to interrupter technique (Rint) and impulse oscillometry (IOS) in discriminating between health and diseases such as asthma and cystic fibrosis using cross sectional or longitudinal baseline measurements in young children.

We were first to develop, implement and validate a simple, convenient and safe indirect bronchial challenge test applying hyper-ventilation with cold air in young children. This test fully substitutes the time consuming and less relevant direct methacholine challenge test. An even simpler and much less expensive method depending simply on dry air hyperventilation was established, although lack of the cooling stimulus seemed to diminish potency. We also provided a soft-share-ware for motivation of hyperventilation on the internet. Under these conditions, a widely disseminated use of indirect challenge testing is possible in young children.

Responsiveness to cold air challenge and bronchodilators as measured with whole-body plethysmography may serve as diagnostic tests for asthma in young children. Bronchial hyperresponsiveness and significant bronchodilator responsiveness do not necessarily coincide.

Whole-body plethysmography proved valid by demonstrating clinically relevant behavior in the assessments of efficacy of classic anti-asthma drugs such as ICS, SABA and LABA, and LTRA during DBPC studies. Applying these tests, we provided for the first time concomitantly objective evidence for the expected efficacy of these drugs in young children, although efficacy was already well proven by studies using parental symptom scoring. We documented reduction in BR from ICS as measured with CACH and sRaw, probably reflecting reduced bronchial inflammation. Moreover, this observation was reflected in reduced symptoms and exacerbations. SABA and LABA were shown to provide a relevant time course of significant bronchodilatation and bronchoprotection against CACH. In-

deed, we were first to provide data to suggest that the initial time course of protection from the LABA formoterol and a SABA was similar. Furthermore, we demonstrated a relevant time course of significant bronchoprotection against CACH with the LTRA montelukast.

We thereby provided means for better judgment of efficacy of anti-asthma medication such as ICS and hopefully provided tools for a more rational asthma management based on phenotyping by the aide of lung function measurement (sRaw) and the assessment of BHR (CACH) in young asthmatic children and children with CF.

## 6. CLINICAL IMPLICATIONS

From the results of our studies and other studies supporting our observations we believe that measurement of sRaw by whole-body plethysmography is ready for every day clinical use in young children with chronic respiratory diseases. Indeed it is a tool comparable to measurements of FEV<sub>1</sub> by spirometry in school children.

Whole-body plethysmography is available at most secondary and all tertiary referral hospitals caring for pediatric patients. Although plethysmographs are built for adults, we have shown that this does not hinder its use in young children, since measurements are feasible, but as previously emphasized, improvements on hardware and software are needed. Measurements of sRaw are highly repeatable, which is a prerequisite for its usefulness for clinical use and research purposes. When used as a routine measurement in a young child suspected of having asthma, a sRaw value of e.g. 2.1 kPa-s (corresponding to a z-score of 4.2), in an otherwise healthy looking child, is highly indicative of asthma, although flow limitation from other reasons should be remembered. When such a measure of sRaw is readily normalized i.e. decreases by more than 25% 20 min after inhalation of a bronchodilator, the diagnosis is almost certainly asthma. The measurement is safe and causes no harm to the young child, since measurements are only performed when children are happy and cooperative.

We have demonstrated a simple test for indirect bronchial challenge, the cold air or the dry air challenge. Though CACH seems preferable, the price may detract from the possibility of it being widely disseminated, and DACH is easily established in any pediatric department or in the collaborating department of clinical physiology. Demonstration of BHR in a child with normal baseline sRaw is certainly indicative of asthma and may therefore be of major help in the diagnostic process in a young child, where diagnosis is otherwise completely dependent on parental history and their reports on treatment effect at each visit. Moreover, repeated measurement of responsiveness to CACH may serve as an objective parameter of asthma control and may guide the clinician to future treatment strategy and adjustment of doses.

Furthermore measurements of sRaw and assessment of BR may serve as useful objective parameters in trials of future therapeutic approaches or new medications for both asthma and cystic fibrosis.

Finally, assessment of lung function (sRaw) and BHR (CACH) may provide a prognostic tool for better estimation of risk of ongoing asthma. However, future studies are needed to provide the evidence.

## 7. FUTURE DIRECTIONS

### *Whole-body plethysmography*

Improvements of equipment and software are needed for more reliable measurement of sRaw in young children.

Improvements of the equipment should pay special attention to the seating and footrest. A face-mask with a large cushion and a built-in non-compressible yet soft tube should be made available.

Software improvements should specifically focus on the algorithms for thermal correction of the inspired volumes. It is not possible at the moment to make decisions on acceptability of resistance loops other than by visual judgement and on discretion of the investigator. The software should include an algorithm for automatic se-

lection of acceptable and rejection of unacceptable loops. Our suggestion to the level of acceptable variability would be close to 10% since this is the CVw% of the test at baseline.

Interactive software with visual prompts should serve as an instructional aide for the child to target the relaxed tidal breathing pattern and the desired breathing rate, and provide biofeedback.

Reference materials should be included in the software of the plethysmograph with allowance for the use of selected yet validated materials. Reporting of measurements should add the z-score.

The protocol on measurements of sRaw proposed in this paper should be further explored. Optimal breathing frequency, number of loops collected and criteria defining the line through the flow-pressure loop need to be further validated, and the best method to reduce the confounding effect of an accompanying adult should be studied; is holding breath equal to slow exhalation?

Further studies in chronic lung diseases such as CF, bronchopulmonary dysplasia, and congenital malformations in the airways are important and feasible with the whole-body plethysmography method since knowledge of lung function in such diseases during preschool age is very limited.

### *Overall considerations on lung function measurements*

Treatment with placebo provided significant bronchodilation in our double-blind study in healthy children (5). This suggests an observer bias in the conduct of all measurements despite a carefully described protocol. Such bias is probably inherent to all lung function measurements including spirometry. Therefore it is recommended that whenever possible the operator should be kept unaware of the history of the patient tested. Furthermore, it is recommended, as was practiced in all studies in this thesis, to have preferable one observer to test the child before and after bronchial stimulation according to the study reporting on inter-observer bias (27), although this wish partly contradicts the wish to blind the observer.

Repeatability is a crucial issue for the use of a lung function test. Long-term repeatability should be determined, and more comprehensive multi-center cross sectional and longitudinal reference values from healthy children of different ethnicity should be collected and would also warrant assessments of repeatability of a lung function test between centers, which is essential for wider dissemination of the test.

### *Bronchial challenge: perspectives specifically to methods and problems raised in this thesis*

Future research should focus on the algorithm used in the decision of hyperventilation level in an individual young child. Specifically we need to study if height would be a better parameter than weight for calculations, e.g. by using the reference material on FEV<sub>1</sub> according to height of young children (105). For a given height of an individual the level of hyperventilation is then calculated as  $25 \times \text{FEV}_1$  predicted from height. This would probably prevent overestimation of hyperventilation level in e.g. an obese child and could improve the overall acceptance specifically in those under age three.

DACH may discriminate between healthy children and asthmatics with a sensitivity equal to CACH, however this has to be properly addressed in a future study including healthy children. In such a study a 4-minute DACH has to be tested versus a 6-minute test.

Although increasing the duration of hyperventilation from 4 to 6 min might have delayed the onset of bronchoconstriction and caused greater bronchoconstriction once the hyperventilation was stopped as previously suggested (124), this was not noticed, but this aspect should be considered in future studies.

Measurement of water and heat loss during hyperventilation tests may allow for further standardization of the methods. Moreover, in order to learn about the exact nature of refractoriness in young children it is necessary to perform repeated CACH with different time intervals.

Repeatability of CACH and DACH in young children needs further



investigation in order to be able to judge treatment effect on BHR in an individual. However, at present it is possible to give a rough judgment by a positive or negative test result of the CACH.

The new version of the software employed to motivate the young child to hyperventilate (3.3.2) has optional facilities for improvement of quality control of the challenge test.

#### General perspectives

We need to investigate how BR to CACH relates to measures of airway inflammation such as NO in exhaled air and condensate of exhaled air in young children.

The relationship between BR to CACH and asthma severity and risk for an exacerbation in young asthmatics should be addressed.

We should assess the value of CACH in the short-term and long-term monitoring of asthma control and treatment in young children. We also need to look at CACH responsiveness as prognostic factor for ongoing asthma beyond preschool age.

### 8. SUMMARY

This thesis is a review of 7 publications based on studies performed in the period from 1995 to 2002 during my appointment as first clinical research fellow and later as consultant and pediatric pulmonologist at the Department of Pediatrics, Clinic I, Pulmonary Service, Rigshospitalet, Copenhagen University Hospital. The work is part of The Copenhagen Prospective Study on Asthma in Childhood (COPSAC).

Longitudinal cohort studies suggest that outcomes such as lung function and bronchial hyperresponsiveness (BHR) in school children and adults with asthma probably are determined already in infancy. Likewise in cystic fibrosis (CF), significant reduction in lung function has been found at a very early age even in the absence of clinically recognized lung disease. The reason for early and longitudinal assessment of lung function and BHR is as obvious as the goals: to enable early diagnosis and intervention, and improve prognosis in asthma and CF. Currently, diagnostics and management of young children with chronic lung diseases rely completely on clinical judgment, implicating risk of inappropriate and useless treatment or no treatment, although needed.

Lung function methods for infants and schoolchildren are not applicable in children between these age groups, i.e. young children 2 to 5 years of age. Three lung function methods have previously qualified as applicable in young children: measurement of specific Resistance of the airways (sRaw) by whole-body plethysmography, Resistance of the respiratory system (Rint) by the interrupter technique and Resistance (R) and reactance (X) of the respiratory system by Impulse Oscillometry technique (IOS). They all assess respiratory mechanics by measuring the relationship between respiratory airflow and the pressure generating this airflow. Focus has primarily been on methodology rather than clinical application. BHR defined as an abnormal increase in airflow limitation following a relevant stimulus to the airways, is a major pathophysiological phenomenon of asthma. Indirect challenges probably induce airflow limitation by acting on inflammatory cells, epithelial cells and nerves, which upon stimulation release mediators or neurotransmitters that induce airway smooth muscle contraction and may better reflect the ongoing airway inflammation in contrast to direct acting pharmacologic tests.

The principal aim of this thesis was to evaluate the clinical application and discriminatory capacity of the mentioned methods as lung function tests in healthy, asthmatics and children with CF. The primary aim was to develop and implement a test for indirect bronchial challenge and assess its validity as judgement of BHR. Moreover the purpose was to compare tests for measurement of baseline lung function and BHR and finally to validate such tests through the studies of documented efficacious anti-asthma medications in asthmatic young children.

376 children participated in the different studies. Principally all

children were 2 to 5 years, but in the CF study the children were 2 to 7 years at inclusion.

Whole-body plethysmography (sRaw) was superior to Rint and IOS in discriminating between health and diseases such as asthma and CF using cross sectional or longitudinal baseline measurements.

We were first to develop, implement and validate a simple, convenient and safe indirect bronchial challenge test applying eucapnic hyperventilation with  $-15^{\circ}\text{C}$  cold air in young children. This test fully substitutes the time consuming and less relevant direct methacholine challenge test. A simpler and less expensive test employing eucapnic hyperventilation with dry room temperature air was also established, however, this test seemed to exhibit diminished responsiveness, probably due to lack of the cooling stimulus.

Responsiveness to cold air challenge (CACH) and bronchodilators as measured by sRaw proved applicable as diagnostic tests for asthma in young children.

sRaw also demonstrated clinically relevant measurements in the assessments of efficacy of classic anti-asthma drugs such as inhaled corticosteroids (ICS), short- and long-acting  $\beta_2$ -agonists (SABA and LABA), and a leukotriene receptor antagonist (LTRA) during double blind placebo-controlled studies. Applying these tests, we provided for the first time concomitantly objective evidence for the expected efficacy of these drugs in young children, although efficacy is already well proven by studies based on parental symptom scoring. BHR was reduced by ICS as measured with CACH and sRaw, probably reflecting reduced bronchial inflammation. Concomitantly we found a reduction in symptoms and exacerbations. SABA and LABA provided a relevant time course of significant bronchodilation and bronchoprotection against CACH. Indeed, we were first to provide data to suggest that the initial time course of protection from the LABA formoterol and a SABA was similar. Furthermore, we demonstrated a relevant time course of significant bronchoprotection against CACH with the LTRA montelukast. Future research should address the relationship between BR to CACH and asthma severity and risk for an exacerbation in young asthmatics. We should also assess the value of CACH in the short- and long-term monitoring of asthma control and treatment in young children, and we need to investigate if CACH responsiveness can serve as prognostic factor for ongoing asthma beyond preschool age.

In conclusion, the research in this thesis provided evidence that sRaw measurement and assessment of BHR by the newly developed CACH method may serve as useful clinical and research tools for earlier diagnosis of asthma and detection of deteriorating lung function in chronic lung diseases, better judgment of efficacy of anti-asthma medication such as ICS and a more rational management based on phenotyping in young asthmatic children and children with CF.

#### LIST OF ABBREVIATIONS

sRaw	specific Resistance of airways by whole-body plethysmography
Raw	Resistance of airways by whole-body plethysmography
Rint	Resistance of airways by the interrupter technique
IOS	Impulse Oscillometry System
FOT	Forced Oscillation Technique
Xrs <sub>5</sub>	Reactance (X) of the respiratory system at 5 Hz
Rrs <sub>5</sub>	Resistance of the respiratory system at 5 Hz
PtcO <sub>2</sub> & PtcCO <sub>2</sub>	transcutaneous Oxygen (O <sub>2</sub> ) & CO <sub>2</sub> tension (P)
SaO <sub>2</sub>	Oxygen (O <sub>2</sub> ) Saturation
MVV	Maximal Voluntary Ventilation
FEV <sub>1</sub>	Forced Expiratory Volume in the first (1) second
FVC	Forced Vital Capacity
PEFR	Peak Expiratory Flow Rate
TGV	Thoracic Gas Volume
MCh	Methacholine Challenge

CACH	Cold Air Challenge
RHES	Respiratory Heat Exchange System
DACH	Dry Air Challenge
CF	Cystic Fibrosis
SD, SDw & SDb	Standard Deviation, SD within subject & SD between subjects
BR & BHR	Bronchial Responsiveness & Bronchial Hyper Responsiveness
BDR	BronchoDilator Responsiveness
ICC	Intraclass Correlation Coefficient
LINMEM	LINear Mixed Effect Modeling
L	Liter
ICS	Inhaled CorticoSteroids
DBPC	Double Blind Placebo-Controlled
PC or PD	Provocative Concentration or Dose, e.g. PD100% <sub>sRaw</sub> is the provocative dose of e.g. histamine causing 100% change in <sub>sRaw</sub> . Sometimes PD100% <sub>HIS</sub> is used as alternative, when response measure is implicit
V'maxFRC	Maximal flow (V') at Functional Residual Capacity (squeeze-jacket)
EIB	Exercise Induced Bronchoconstriction
SABA & LABA	Short- & Long Acting Beta-2-Agonists
LTRA	LeukoTriene Receptor Antagonist

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