Application and Interpretation of Paediatric Lung Function Tests in Health and Disease

Jane Kirkby

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Portex Unit of Paediatric Anaesthesia, Pain Research, Critical Care, Respiratory Medicine, Physiology and Physiotherapy.
UCL, Institute of Child Health

Declaration

I, Jane Kirkby, confirm that the work presented in this thesis is my own.
Where information has been derived from other sources, I confirm that this has been indicated within the thesis.

Abstract

Background: Lung function tests (LFT) provide valuable insights into respiratory physiology, and have been proven to be useful outcome measures in both clinical research and the clinical management of children with lung disease. Standardised methods and appropriate interpretation are, however, essential if these measurements are to be applied reliably. The aims of this thesis were to improve the application and interpretation of LFT's in children and to determine the extent to which ethnic differences in lung function (LF) occurred between healthy Black and White children after adjusting for height, sex and age.

Methods: A series of investigations using four commercially available LFT (Impulse oscillometry (IOS), specific airways resistance (sR_{aw}), plethysmographic lung volumes, and spirometry) involving 400 healthy children (214 Black and 186 White) aged 4-12y were undertaken. Upon determining the most appropriate methods for interpreting LF in health, the LFT's and a respiratory health questionnaire were applied to children with Sickle Cell Disease (SCD) to determine the extent to which each outcome measure identified LF abnormalities in these children.

Results: Reference data for measurements of sR_{aw} in children were developed as well as recommendations for interpreting spirometry and plethysmography in Black children. Despite the relatively high proportion of respiratory symptoms reported in SCD, the proportion of children with LF results falling outside the limits of normal was relatively small, and a pattern of restrictive lung disease was observed. Of the outcomes assessed in this thesis, spirometry appeared to be the most robust outcome measure for routine assessment of LF in SCD.

Conclusions: Results from this thesis contribute to the literature that SCD is primarily associated with restrictive lung disease. Furthermore, the new interpretation strategies developed during the work for this thesis prevented significant misinterpretation of LF in Black children, and improved the standards for using these LFT's in children.

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List of abbreviations

ACS:	Acute Chest Syndrome
ATS:	American Thoracic Society
AX:	Integrated Area of Reactance
BEV:	Back Extrapolated Volume
BD:	Bronchodilator
BDR:	Bronchodilator Response
bpm:	Breaths per minute
CDC:	Centre for Disease Control
CF:	Cystic Fibrosis
CV:	Coefficient of Variation
DL_{CO}:	Diffusing Capacity of the lung for carbon monoxide
E:	Elastance
EOTV:	End of Test Volume
eNO:	Exhale Nitric Oxide
ERS:	European Respiratory Society
ERV:	Expiratory Reserve Volume
Fdr:	Frequency Dependence
FEF:	Forced Expiratory Flow
FET:	Forced Expiratory Time
FEV_1:	Forced Expired Volume in one Second
FFT:	Fast Fourier Transformation
FRC:	Functional Residual Capacity
FOT:	Forced Oscillation Technique
F_{res}:	Resonant Frequency
FVC:	Forced Vital Capacity
GLI:	Global Lungs Initiative
Hb:	Haemaglobin
HB:	Healthy Black
IC:	Inspiratory Capacity
ICH:	Institute of Child Health
ILD:	Interstitial lung disease
IOS:	Impulse Oscillometry
LF:	Lung Function
LFT:	Lung Function Test
LLN:	Lower limit of normal

MBW:	Multiple Breath Washout
MDI:	Metered Dose Inhaler
MOP:	Manual of Procedures
P:	Pressure
P_{amb}:	Ambient pressure
P_{alv}:	Pressure in the Alveoli
P_{atm}:	Atmospheric pressure
P_{mouth}:	Pressure measured at the mouth
P_{pl}:	Pleural pressure
PEF:	Peak Expiratory Flow
PIF:	Peak Inspiratory Flow
PRN:	Pseudo Random Noise
QC:	Quality Control
R:	Resistance
R_{aw}:	Airways Resistance
R_{int}:	Resistance measured by Interrupter technique
$R_{lung:}$	Pulmonary resistance
R_{rs}:	Resistance of the total respiratory system
R_5:	Resistance at oscillation frequency of 5Hz
R_{10}:	Resistance at oscillation frequency of 10Hz
R_{15}:	Resistance at oscillation frequency of 15Hz
R_{20}:	Resistance at oscillation frequency of 20 Hz
RV:	Residual Volume
SAC:	Sleep and Asthma Cohort Study
SCD:	Sickle Cell Disease
SD:	Standard Deviation
SEM:	Standard Error of the mean
SLIC:	Size and Lung Function Study
sR_{aw}:	Specific Airways Resistance
sR_{eff}:	Effective Specific Airways Resistance
sR_{tot}:	Total Specific Airways Resistance
TGV:	Total Thoracic Gas volume
TLC:	Total Lung Capacity
UCL:	University College London
ULN:	Upper limit of normal
V':	Flow
V'':	Flow acceleration

X_{rs}:	Reactance of the total respiratory system
X_5:	Reactance at oscillation frequency of 5Hz
X_{10}:	Reactance at oscillation frequency of 10Hz
Zrs:	Impedance of the total respiratory system
95% LA:	95% Limits of Agreement
95% CI:	95% Confidence Interval

Summary of publications and awards related to the thesis

Papers:

- The EPICure study: Comparison of pediatric spirometry in community and laboratory settings. *Pediatric Pulmonology* 2008; 43(12):1233-1241. **Kirkby J**, Welsh L, Lum S, Fawke J, Rowell V, Thomas S, Marlow N, Stocks J
- Reference equations for specific airway resistance in children: The Asthma UK Initiative. *European Respiratory Journal* 2010; 36(3):622-629. **Kirkby J**, Stanojevic S, Welsh L, Lum S, Badier M, Beardsmore C, Custovic A, Nielsen K, Paton J, Tomalak W, Stocks J
- Interpretation of pediatric lung function: Impact of ethnicity. *Pediatric Pulmonology* 2012; 10.1002/ppul.22538. [Epub ahead of print]. **Kirkby J,** Bonner R, Lum S, Bates P, Morgan V, Strunk RC, Kirkham F, Sonnappa S, Stocks J

Letters:

- "Stitching and switching" The impact of discontinuous lung function reference equations. *European Respiratory Journal.* 2012; 39 (5). **Kirkby J,** Aurora P, Spencer H, Rees S, Sonnappa S, Stocks J
- The Global Lungs Initiative: Not JUST another Reference Equation. *Inspire.* 2011; 11(2):12-14. **Kirkby J,** Stanojevic S, Stocks J

Abstracts:

- Spirometry at 11 years after extremely preterm birth. *American Journal of Respiratory and Critical Care Medicine.* 2008; 177, A558. **Kirkby J**, Welsh L, Fawke J
- Upgrades of spirometry software: Are results comparable? *European Respiratory Journal.* 2008; 32[Supplement 52], 552s. **Kirkby J**, Welsh L, Stocks J
- Is the clinical application of specific airway resistance in young children limited by inter-centre differences? *European Respiratory Journal.* 2009; 34[supplement 53], P1178. **Kirkby J**, Stanojevic S, Welsh L, Custovic A, Tomalak W, Badier N, Nielson K, Stocks J
- The role of respiratory follow up in children with sickle cell disease. *European Respiratory Journal.* 2010; 36[Supplement 54], P4747. **Kirkby J,** Baba I, Kirkham F, Roberts I, Roberts-Harewood M, Warner J
- The influence of breathing pattern on specific airways resistance. *Thorax* 2010; 65[Supplement IV], A131-A132. **Kirkby J**, Sonnappa S, Stocks J
- Interpretation of plethysmography in healthy young children. *Thorax* 2011;66[supplement IV], A122-123. **Kirkby J,** Bonner R, Lum S, Bates P, Morgan V, Strunk R, Kirkham F, Sonnappa S, Stocks J

Awards:

- 2009 UCL, Institute of Child Health Poster Competition: Specially Commended in the Year 1 Category. Poster entitled: *A Multi-centre comparison of specific airways resistance in young children*
- 2010 UCL, Institute of Child Health Poster Competition: 1st Prize in the Year 2 Category. Poster entitled: *Influence of breathing pattern on specific airways resistance*
- 2011 UCL, Institute of Child Health Poster Competition: 1st Prize in the Year 3 Category. Poster entitled: *Interpretation of plethysmography in healthy young children*
- Asthma UK Travel award to attend the 2009 European Respiratory Society Congress

1 Introduction

1.1 Aim of the thesis

Lung function tests provide valuable insights into respiratory physiology, and have been proven to be useful outcome measures in clinical research as well as valuable adjuncts in the clinical management of children with lung disease. Standardised methods and appropriate interpretation are however, essential if these measurements are to be applied reliably. The broad aim of this thesis is to evaluate the methods and interpretation of paediatric lung function measurements in children aged 4 to 12 years. Prior to considering this aim, this introduction chapter will briefly review basic respiratory physiology in health and disease; evaluate the literature on the methods of assessing and interpreting lung function in children (at inception of this thesis in 2008), and identify the lung function tests selected for evaluation in this thesis. This chapter will conclude with a summary of the different methods of interpretation, the aim of the thesis and the hypotheses.

1.2 Respiratory physiology

The principle function of the lungs is to transport oxygen from the atmosphere to the lungs and to release carbon dioxide from the bloodstream into the atmosphere. The structure of the lung is perfectly designed for this function; during inspiration, air flows into the lung as a consequence of pressure changes within the lung (brought about by the action of the "respiratory pump," that is, the diaphragm, intercostal and accessory muscles, operating on the thoracic cage). While expiration is generally a passive process measures of forced expiration can provide information about the mechanical properties of the lung. This section will describe the functional anatomy of the lung, respiratory mechanics, and forced expiratory flows in health and lung disease.

1.2.1 Functional anatomy

The respiratory system comprises the upper airways (the nasal cavity, pharynx and larynx), the lower airways (trachea, primary bronchi and bronchial tree) and the small bronchioles and alveoli within the lung tissue (Figure 1-1a). Each alveolus is closely associated with a network of capillaries. Expansion of the lungs (i.e. inspiration) affects all its components, but mainly increases the surface area of alveoli and, in doing so facilitates the exchange of gas across the alveolar-capillary membrane.

Figure 1-1: a) Anatomy of the respiratory system. b) muscles used for ventilation
Adapted from "Human Physiology" by Dee Unglaub Silverthorn. **ISBN10:** 0321590899

The main muscles involved in breathing are shown in Figure 1-1b. Breathing is largely driven by the diaphragm, which is innovated by the phrenic nerve. Upon contraction, the diaphragm descends downwards pulling the ribcage with it. Concurrently the external intercostal muscles contract and the ribcage rises, thus the thorax is elongated and widened. Since the lungs are connected to the thoracic wall by the pleural membranes they also expand. This results in increasing the volume, and decreasing the pressure within the lungs, causing air to flow into the airways along the pressure gradient. The “mechanical work” required to expand the lungs, and overcome the impedance to movement of the lungs, chest wall and abdomen is described in section 1.2.3.

The framework of the lungs is made up of bundles of elastic and collagen fibres that extend from the large airways down to the alveoli and across to the pleura and blood vessels. The constituent fibres are relatively indistensible but they can move in relation to each other so the bundles of fibres lengthen and uncurl and alter their relative positions. The force required to maintain inflation of the lung is provided by the chest wall and diaphragm, which are in turn recoiled inward by the pull of the lungs. The lung–chest system thus acts as two opposed coiled springs, the length of each of which is affected by the other, and ultimately determines the lung volume.

1.2.2 Determinants of lung volume

The amount of gas in the lungs at different levels of inflation is represented as volumes when consisting of single components, and capacities when they comprise two or more components. The partitioned lung volumes are labelled in the spirogram (Figure 1-2).

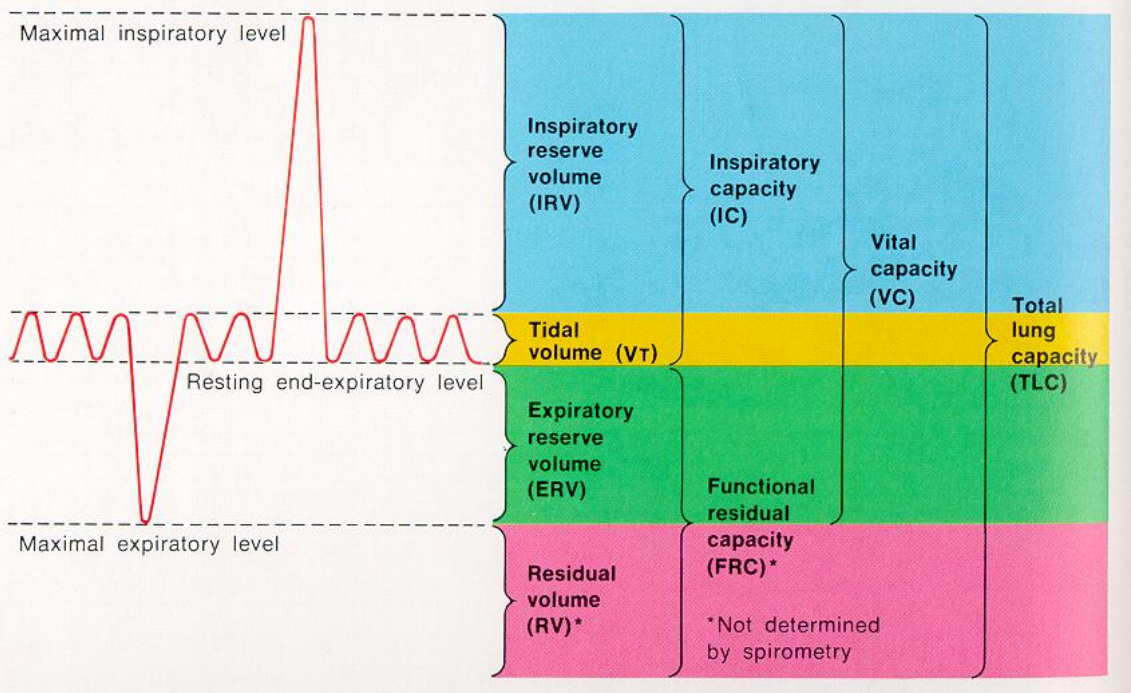

Figure 1-2: Spirogram identifying different lung volume levels.
Legend: IRV = Inspiratory Reserve Volume; V_T = Tidal Volume; ERV = Expiratory Reserve Volume; IVC = Inspiratory Vital Capacity; RV = Residual Volume; IC = Inspiratory Capacity; FRC = Functional Residual Capacity; TLC = Total Lung Capacity

Lung volumes are governed by a balance of forces of different mechanisms relating to respiratory muscle activity, chest wall function, airway size and tone, and the elastic recoil properties of the lung. In the absence of movement, the extent of lung inflation reflects a balance between the elastic recoils of the lungs and chest wall, gravitational force and tension in the respiratory muscles. This “resting lung volume” is termed Functional Residual Capacity (FRC) and occurs when the inward pull of the lung is balanced by the outward pull of the chest wall. Movement occurs when the equilibrium is disturbed and is influenced by the strength of the applied force, the elasticity of the lung and chest wall, the resistance to movement and the inertia of the thoracic cage, lung tissue and volume of gas contained in the lung. At Total Lung Capacity (TLC) and Residual Volume (RV) the inspiratory and expiratory muscles have reached the limit of their ability to oppose the elastic recoil. These concepts are described in detail in the following section (section 1.2.3).

1.2.3 Respiratory mechanics

The mechanical work required to ventilate the lungs is expressed over an entire breathing cycle comprising inspiration and expiration. Air is moved in or out of the lungs whenever the sum of the pressures developed by the passive recoil of the respiratory system and by the respiratory muscles is different from zero. Work is required to expand the lungs and overcome the impedance to movement of the lungs, chest wall and abdomen. To produce air movement, the driving pressure of the respiratory muscles must overcome the forces opposing the movement of gas flow. The mechanics of the respiratory system are governed by the following forces:

1.2.3.1 Impedance

Impedance (Z) in electronics terms is a measure of the overall opposition of a circuit to current, in other words: how much the circuit impedes the flow of current. The concept of impedance with respect to the lungs is the total opposition to breathing/airflow, and represents the net sum forces that must be overcome to generate flow which encompass the resistive, inertive and the visco-elastic forces that oppose respiration.

1.2.3.2 Resistance

Resistance (R) means opposition to movement and is caused by the forces of friction that occurs between the gas molecules within the airways. In general it represents the sum of viscous resistances of which airway resistance is the most significant. Measurement of resistance to flow of air is calculated by Ohm's Law which states: **Resistance is equal to pressure (P) divided by flow (V').**

Resistance is a familiar parameter in conventional pulmonary function, and is described further in section 1.2.5.

1.2.3.3 Inertance

Inertance (I) is the force required to overcome the inertia (the tendency of an object to resist a change in motion) of the lungs and acceleration of gas (volume of air) into and out of the lung. It therefore describes the relationship between pressure and volume acceleration (speed of gas flow).

1.2.3.4 Elastance

During inspiration, contraction of respiratory muscles stretches the elastic and collagen tissue network of the lungs and pleura. The work that is done in stretching the lung is not however, dissipated as heat, instead it is stored in the structures which have been stretched, and used to drive the subsequent expiration (i.e. by recoiling back to previous volume). The force required to overcome the elastic properties to expand the

lung during inspiration is termed elastance (E), and is directly related to lung volume. As the lung volume increases during inspiration, the elastic recoil pressure of the lung (P_{el}) increases. The reciprocal of elastance (1/E) is the compliance (C) of the lung and represents the change in volume per unit change of pressure. P_{el} can be regarded as the driving force of the expired gas since it overcomes the friction in the airways and the remaining pressure is used to accelerate the expired gas.

Figure 1-3 illustrates the interactions between the elastic properties of the lung and chest wall. At FRC the elastic recoil of the lung (pulling inwards) is balanced by the elastic recoil of the chest (pulling outwards), thus the transmural pressure is 0 cmH_2O (0 kPa). At every lung volume, the overall transmural pressure is the sum of the lung and chest wall pressures measured separately. The compliance (determined by the volume change for a particular pressure change) of the respiratory system (lung+chest wall) is large at low/moderate lung volumes (i.e. large volume changes occur as a result of small increases in pressure), but low at high lung volumes (i.e. large pressure changes cause minimal volume changes) (Figure 1-3).

Figure 1-3: Pressure-volume curve of the lung and chest wall.
Legend: Elastic properties of the lung and chest wall can be examined by determining pressures over the entire range of volumes that the lung can contain. The elasticity of the lung and chest wall are opposing forces, and the overall compliance of the lungs is dependent upon the lung volume. Unit conversion: 1cmH_2O = 0.1kPa.
Illustration adapted from: “Respiratory Physiology” by West (ISBN: 0683089374)

1.2.4 Pressure changes throughout the breathing cycle

Airflow occurs as a consequence of pressure changes throughout the breathing cycle as illustrated in Figure 1-4.

Figure 1-4: Alveolar and Intrapleural pressure changes during the respiratory cycle.
Legend: Pressures given in kPa. P_{pl} remains negative throughout the breathing cycle, whereas $P_{alv} = P_{atm}$ when there is no flow (end-expiration (a & e) and end-inspiration (c)), but is negative during inspiration (b) and positive during expiration (d).
Illustration adapted from "Infant respiratory function testing" by J Stocks *et al*. ISBN: 0471076821

During inspiration, the volume of the lung increases, hence the pressure within the alveoli (P_{alv}) reduces. Since P_{alv} is more negative than atmospheric pressure (P_{atm}), a driving pressure is established and airflow occurs along the pressure gradient. The increase in volume is brought about partly by contraction of the diaphragm, and partly by the action of the intercostal muscles. Since the lung is elastic, during passive expiration the lungs and chest wall return to their original state, thus decreasing the volume of the lung and increasing the pressure within the lungs, such that P_{alv} is greater than P_{atm} and air moves out of the lungs. At the end of expiration or end of inspiration $P_{alv} = P_{atm}$ hence there is no flow and P_{atm}, and/or pressure measured at the airway opening (P_{mouth}) is zero.

Pleural pressure (P_{pl}) is the pressure difference between inside and outside the lung (within the pleural space), and is generally negative (in relation to P_{atm}) due to the elastic recoil of the lungs. P_{pl} increases (i.e. becomes more negative) as the lung is stretched and its volume increases during inspiration. During the expiratory phase of the respiratory cycle, the inspiratory muscles relax and P_{pl} becomes less negative with respect to $P_{atm.}$ The elastic recoil pressure of the lung tissue then compresses the alveolar gas and raises its pressure above that at the mouth (P_{mouth}) and air flows out of the mouth or nose.

The continuous pressure changes throughout the breathing cycle are intrinsically related to the mechanical properties of the lung. Knowledge of these pressure changes allow us to make measurements and assumptions about the function of the lungs.

1.2.4.1 Phase relationship of acceleration, flow and volume

The principles of respiratory mechanics are often based on the assumption of a linear behaviour of the pulmonary-thoracic system and are described in relation to flow, volume and acceleration under defined physiological conditions. However, changes in flow are in fact the direct result of pressure changes within the system and not synchronous with time. During inspiration, the fall in P_{alv} precedes airflow, and volume is maximal at a time when flow is zero. Hence there is a phase (timing) difference between flow (V') and pressure (P) with the pressure leading the flow. This phase difference is determined by the elastic and the inertive properties (the resistance to acceleration of a volume of gas) of the lung (Figure 1-5).

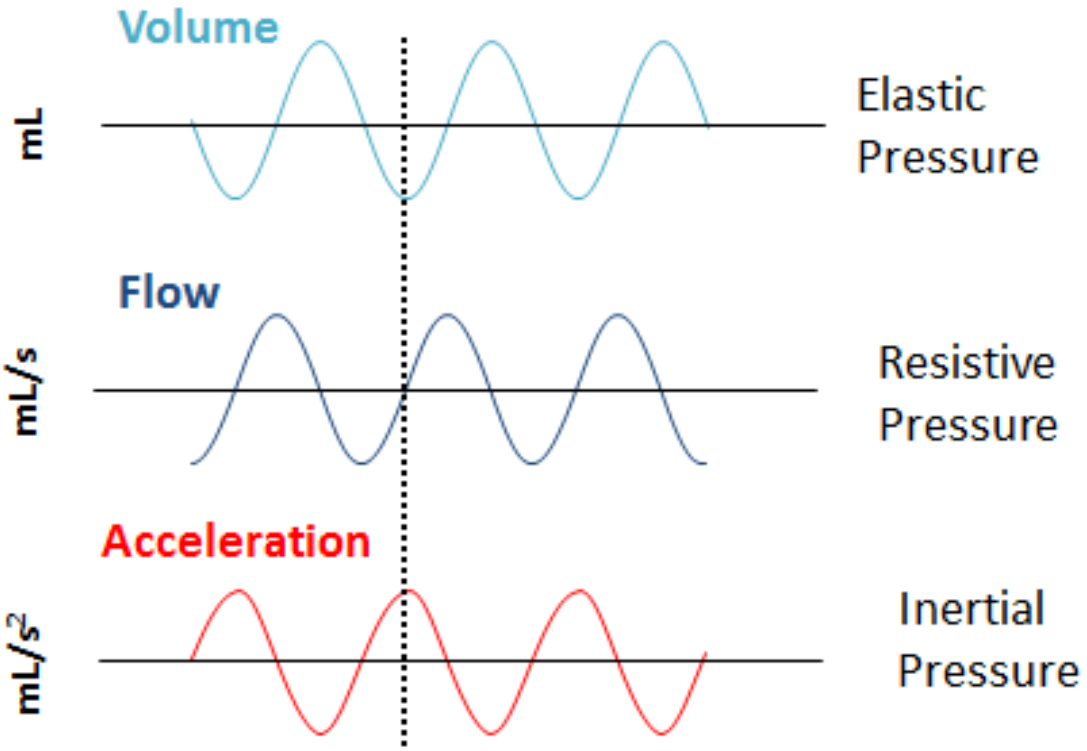

Figure 1-5: Phase relationship between volume, flow and acceleration.
Legend: 1) Elastic forces are overcome to achieve lung expansion and hence increase in lung volume. 2) The change in lung volume creates a pressure gradient in which flow can occur, however resistive forces have to be overcome to allow airflow. 3) Simultaneously inertial forces (the force acting against the acceleration of a mass of air) are overcome.

Figure 1-5 illustrates the phase relationship between volume flow and acceleration. Inspiration is initiated by contraction of the diaphragm and intercostal muscles, which are the driving force to overcome the elastic forces within the lung. Lung volume increases thereby creating a pressure gradient between the airway opening (which is equal to P_{atm} (0 kPa)) and the alveoli (P_{alv}, which is negative pressure with respect to P_{atm}). Airflow occurs along the pressure gradient once it has overcome the forces opposing the movement of gas flow: i.e. the resistance offered by the airways to the flow of gas, and by the viscous properties of the tissues resisting their deformation, and by the inertia of the gas and tissues which undergo acceleration. If a line is drawn through the point of zero flow, it will intersect with the trough of the volume waveform and the peak of the acceleration waveform (Figure 1-5). Thus it can be seen that flow leads volume by a ¼ of a cycle or 90^0 and that it lags acceleration by the same amount. Volume and acceleration are opposite in phase (or 180^0 *out of phase*) with each other. The various components of resistance, both in-phase with flow (also termed “real”) and out-of-phase with flow (also termed “imaginary”) and pressure changes throughout the breathing cycle are described in section 1.2.3 and can be measured with specialised lung function tests (described in section 1.5).

1.2.5 Airways resistance

The unique structure of the lungs facilitates gas exchange. The progressively subdividing system of bronchi and bronchioles offers resistance to flow, which is essential to allow adequate time and surface area for gas exchange, in addition to allowing heating and humidification of air in to the lungs. Measurement of resistance to flow of air is calculated by Ohm's Law which states:

Resistance is equal to pressure (P) divided by flow (V').

The resistance to the flow of air into and out of the lung is determined by Poiseuille's Law which states: $\mathbf{R \alpha 8nl/\pi r^4}$

Where n= gas viscosity

l= length of the tube

r = radius of the tube.

Poiseuille's Law states that in a straight circular tube resistance is proportional to the length of the tube and inversely proportional to the fourth power of the radius. This means that if the length of the tube is halved, the resistance is halved, however if the radius is halved, the resistance would increase by 16 fold. As the airways get progressively narrower, the resistance within the individual airways increases, however the number of airways increases exponentially, such that the cross-sectional area of the lungs increases towards the periphery where overall resistance decreases (Figure 1-6).

Figure 1-6: Cross-sectional area and subsequent airways resistance
Illustration adapted from: "Respiratory Physiology" by West (ISBN: 0683089374)

In the presence of lung disease, resistance in the peripheral airways (defined as bronchioles <2mm in diameter) is likely to increase due to significant peripheral airway oedema, inflammation, secretions and/or bronchoconstriction, such that airways resistance in the peripheral airways increases relative to the central airways.

"Resistance" can be divided into three categories:

Airways resistance (R_{aw}): Relates to changes in alveolar pressure (P_{alv}) to airflow. Since during normal breathing frequencies, the pressures producing acceleration of flow are negligible, flow resistance of the airways may be measured by recording the pressure difference between appropriate points (e.g. at end inspiration or end expiration) in time simultaneously with airflow. Measurement of airways resistance is discussed further in section 1.7.

Pulmonary (lung) resistance (R_{lung}): Comprises the lung tissue resistance plus the airways resistance. Lung tissue resistance refers to the resistance offered by the lung tissue as it expands, and varies with lung volume: The airways are surrounded by and attached to the alveoli. At high lung volumes, the alveoli are more distended and the elastic recoil tension in their walls is higher, thus the lumen of the airways are pulled open by the high tension in the distended alveolar walls. The increased airway calibre results in a reduction in airways resistance (R_{aw}) and resistance within the airways is decreased.

At lower lung volumes, the elastic recoil tension in the alveolar wall is less and thus reducing airway calibre in turn contributes more to airway resistance. Pulmonary resistance is calculated from changes in transpulmonary pressure.

Total respiratory resistance (R_{rs}): Comprises the total resistance (airway resistance + lung tissue resistance + chest wall resistance) to air flow in and out of the respiratory system. It includes the inertia of the respiratory system, the tissue resistance of the lungs and chest wall, and airways resistance.

1.2.5.1 Impact of flow on airways resistance

The principles of airways resistance are based on Poiseuille's Law which is based on "straight circular tubes." The lungs however are a series of branching tubes, in which the pressure difference depends on the rate and pattern of flow (Figure 1-7).

Figure 1-7: The different types of flows that can be generated.
Legend: A) Laminar flow: flow is stream lined and parallel to the sides of the tube. B) Transitional flow: unsteadiness in the flow, especially at the branches. C) Turbulent flow: Complete disorganisation of stream lines.
Illustration adapted from: "Respiratory Physiology" by West (ISBN: 0683089374)

Resistance can only be described as linearly increasing pressure with increasing flow as long as the flow type is laminar (which occurs at low flow). At high flows (turbulent flows) there is complete disorganisation of stream lines and resistance may be described in relation to **Rohrer's law:** $\Delta P = k_1 . V' + k_2 . V''$

Rohrer's law describes airflow in the airways during tidal breathing from laws on fluid mechanics and states that: The change in pressure is equal to a constant (K_1) multiplied by flow (V') plus another constant (K_2) multiplied by flow acceleration (V''). It suggests that pressure changes become more dependent on gas density (rather than viscosity) and V'' (rather than flow (V')) and implies that R_{aw} will increase as flow increases e.g. on exercise, due to increased turbulence. This implies that breathing pattern has important implications when measuring $R_{aw.}$

1.2.6 Forced expiratory flow

Quiet expiration is a passive process due to the elastic properties of the lungs. However forced expiration is an active process requiring contraction of expiratory muscles (oblique and transverse abdominus muscles, internal intercostals, and the latissimus dorsi). During a forced expiration the pressure distribution inside the bronchial tree depends on lung volume, gas properties, flow, and airway calibre. Thus the mechanics of the airways and that of airflow are closely linked. Measurements of forced expiratory flow are thought to reflect the integrated mechanical properties of the lungs and airways.

Analysis of both flow-volume and pressure-flow curves gives important indications on the physiology of the lungs. The maximum expiratory flow-volume (MEFV) curve has characteristic features that reflect the properties of the lungs. During inspiration, flow is maximal at mid-volume. It varies directly with the applied pleural pressure, therefore it is effort dependent. During maximal expiration from a position of full inspiration the flow rises to a peak early in the manoeuvre (generated mainly by the expiratory muscles), and then exhibits a gradual decline as the volume in the lungs diminishes (dependent mainly on elastic recoil, and not from expiratory muscles) (Figure 1-8). The relationship of flow to time during inspiration and expiration is therefore different, with differing shapes on the inspiratory and expiratory flow-volume curve.

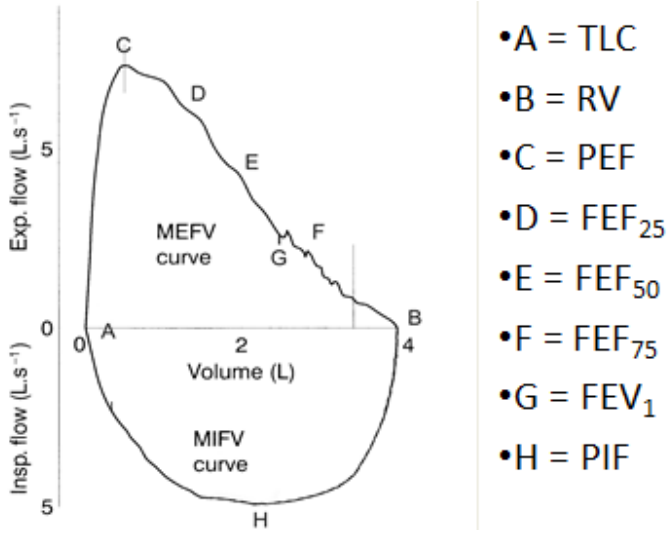

Figure 1-8: Flow-Volume curve during maximal expiration and maximal inspiration.

MEFV curves are largely reproducible, because, beyond a critical level of effort, the expiratory flow is independent of P_{pl}. This independence of effort, taken together with the observed dependence of maximum flow on lung volume, suggests that there is a lung mechanism which limits the expiratory flow (see section 1.2.7.1).

1.2.7 Airflow limitation

Airflow limitation is the condition in which, at a given lung volume, flow is independent of the pressure difference between the airway opening and the alveoli (assuming the pressure difference is sufficiently large to allow maximal flow). The difference extends to the relationship of flow to applied pressure and is demonstrated with the iso-volume pressure-flow curve.

1.2.7.1 Iso-Volume Pressure-Flow (IVPF) curve

An iso-volume pressure-flow (IVPF) curve can be constructed by repeating inspiratory and expiratory flow manoeuvres at gradually increasing effort (greater P_{pl} and P_{alv}) and recording flow, P_{alv} and the total thoracic gas volume (TGV) (Figure 1-9). Intra-thoracic pressures are measured with an oesophageal catheter and are plotted against expiratory flows measured at the mouth at specific lung volumes. The IVPF curve demonstrates that, with the exception of the highest lung volumes, expiratory flow becomes limited or effort independent at relatively modest positive intra-thoracic pressures.

Figure 1-9: Iso-volume pressure flow curve
Legend: Repeated maximal inspiratory and expiratory flow-volume curves at gradually increasing effort (greater P_{pl} and P_{alv}) and recording flow, alveolar pressure and TGV, demonstrates that there is a plateau of flow even with increasing effort (increased driving pressure) so the lung limits flow (not respiratory muscles). Flow therefore said to be effort independent. Illustration adapted from “Physiology and Practice of Pulmonary Function” by Mike Hughes.

The pressure-flow relationship at a constant lung volume is informative: At the start of expiration a rise in P_{pl}, together with the positive recoil pressure of the lungs, increases P_{alv} and initiates expiratory flow. However once a threshold pressure has been generated, a further increase in pressure does not augment the flow (seen by the plateau on the IVPF curve (Figure 1-9). The existence of flow limiting mechanisms has the effect that beyond a threshold level of effort the maximal expiratory flow reflects mainly the intrinsic mechanical properties of the lungs, not expiratory effort. These mechanical properties can be described further by the wave speed theory and the development of the equal pressure point (EPP) or choke point.

1.2.7.2 Wave speed theory

Wave speed (velocity) is the maximal speed at which a pulse wave of gas molecules can be transmitted along an airway and is calculated by the formula:

$$\mathbf{V'_{ws} = [1/\delta \cdot (\Delta P_{transmural}/\Delta A) \cdot A]^{1/2} \cdot A}$$

Where: A = cross-sectional area

V'_{ws} = Wave speed

δ=gas density

$\Delta P_{transmural}$ = transmural pressure difference

ΔA = change in cross-sectional area.

Thus, the less dense the gas, the stiffer the tube wall, and the larger its cross-sectional area, the higher the wave speed and hence, the higher the flow that can theoretically pass the tube. According to this theory, for compressible tubes such as the airways, the maximum velocity is determined by an interaction between the velocity, the compliance of the airway wall and the convective acceleration of the gas. The maximal flow therefore is the product of the velocity and the cross-sectional area of the tube, and the mean flow cannot exceed the speed at which the pressure driving the flow is propagated along the tube.

The lungs consist of a series of airways each with their own wave speed flows that depend on a number of factors and vary with lung inflation. Flow limitation will occur at the point along the bronchial tree at which local wave speed flow is minimal for a given lung volume and this point is called the equal pressure point (EPP) (or choke point). At this location, airflow can increase until local wave speed flow is reached, however if more energy is added (more effort exerted), it may be converted to noise (wheeze), but not to increase flows.

1.2.7.3 Equal pressure point

During forced expiration, the driving pressure forcing flow from the lungs is the sum of the pressure actively applied to the pleural space plus the lung recoil pressure. Throughout forced expiration, flow-related frictional and convective accelerative intrabronchial pressure losses occur. At some point along the airways downstream from the alveoli to the mouth, flow-related pressure losses will equal the elastic recoil pressure and the difference between intrabronchial and extrabronchial pressure losses will be zero. This point is referred to as the equal pressure point (EPP). Distal to this point, further intrabronchial airway pressure losses result in lower pressures within the airways than around them and leads to dynamic compression of downstream airways (i.e. towards the mouth).

During forced expiration, EPPs move due to changes to the many factors that determine wave speed flow. At high lung volumes, the level of the bronchial tree with the smallest total cross-sectional area is in the trachea. In the smaller airways, the cross-sectional area is large at high lung volumes and decreases steadily as forced expiration proceeds to lower lung volumes. Peripheral airway resistance thus increases steadily with decreasing lung volume. Both increases in resistive losses and declining lung elastic recoil pressure diminish intra-airway pressures as exhalation proceeds. As a result, the EPP and the location where airways are dynamically compressed move toward the alveoli as lung volume decreases. Thus, the site of flow limitation during a forced expiratory manoeuvre occurs initially in the trachea or central airways in normal subjects and moves progressively upstream towards the alveoli as forced exhalation proceeds.

An increase in resistance in the peripheral airways (e.g. due to inflammation) exaggerates the flow limiting mechanism because it magnifies the pressure drop along the airways. Low lung volume and reduced recoil pressure (e.g. loss of elasticity, such as emphysema) would also exaggerate the flow-limiting mechanism (Figure 1-10).

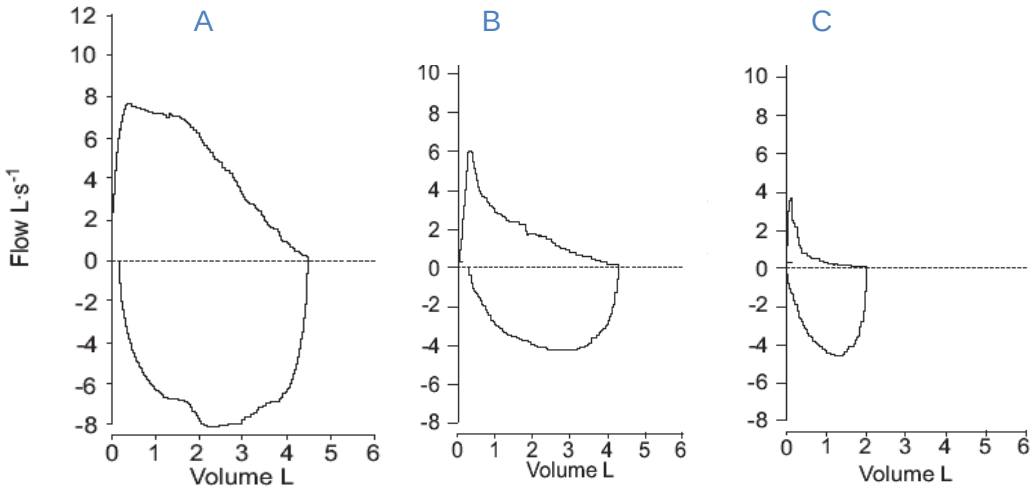

Figure 1-10: Maximal Flow-Volume curves.
A) Health, B) moderate airway obstruction, C) severe airway obstruction
Legend: Reduction in forced expiratory flows are indicative of airways obstruction. In severe airways obstruction (C) a reduction in vital capacity also occurs due to the airway closure.

In summary, maximal flow-volume curves have a distinctive shape which provides important information regarding airway mechanisms and airflow limitation. On inspiration, no intrapulmonary flow limitation develops as the airways are dynamically distended by the negative P_{pl}. The entire MIFV curve and the upstroke of the MEFV curve are therefore dependent on the speed and force of contraction of the expiratory and inspiratory muscles and the flow resistance at each lung volume. At the start of expiration however, the speed of airflow is limited by the rate at which the expiratory muscles are able to contract, until wave speed flow is reached and flow limitation is established. Flow is then limited and is independent of effort.

1.3 Lung function in health

Lung function in health is significantly influenced by a number of factors including age, body size, genetic factors such as sex and ethnicity, socio-economic factors, family history of asthma and atopy, and environmental exposures including cigarette smoke.[1,2] The previous section (1.2) has described the structure and function of the lung and the physiology of respiration. A comprehensive understanding of lung physiology and knowledge of normal growth and development of the lung in childhood is essential if lung function tests are to be interpreted appropriately.

1.3.1 The growing lung

While the function of the lungs remains the same throughout life, it is important to note that children's lungs are not simply smaller versions of adult lungs. Significant structural and functional changes occur with growth primarily due to the different patterns of airway and parenchymal development (dysanaptic growth). The full adult compliment of conducting airways and a blood-gas barrier sufficient to sustain life is present by 27 weeks gestation; however the airways and gas-exchanging surface area continue to develop after birth and throughout early childhood. This rapid alveolar development stage increases lung volume at a greater rate than airway development. Evidence of this dysanaptic growth can be seen in Figure 1-11 where the FEV_1/FVC ratio (an indication of airway dimension relative to lung volume) decreases with age. The "kink" occurring in puberty is thought to be related to differing changes in stature and muscle strength in relation to the pubertal growth spurt.

Figure 1-11: The decline in FEV_1/FVC throughout life.
Adapted from Quanjer *et al*, ERJ 2010[3]

During early childhood there is an almost linear lung and airway growth pattern, where alveolar expansion, airway elongation and enlargement occur at a similar rate. By the start of puberty lung function is equal in males and females of equal height, however by the end of puberty lung function in males is up to 25% higher than females of the same height. These differences relate to differences in the growth of the thoracic cage and respiratory muscle strength. Lung growth stops when the thoracic cage stops growing, and during adulthood there are further structural changes whereby the elastic recoil of the lung reduces with advancing age, and the lungs are more compliant.

The continuous structural changes of the lungs throughout life have important implications. Figure 1-12 depicts the increase in lung growth throughout childhood, and the steady decrease after the peak lung function at around 21-25 years of age. When measuring lung function, age and growth need to be taken into account when interpreting lung function results.

Figure 1-12: Changes in FEV_1 occurring throughout life.
Legend: There is a steady increase in FEV_1 throughout childhood which peaks at around 21 years for males (~18 years for females), after which there is a steady decline.
Adapted from Quanjer *et al*, ERJ 2010[3]

Growth and age play a key role in determining lung volumes. During childhood, in addition to the overall growth of the lungs and thorax, there are significant changes in functional anatomy which needs to be considered when interpreting partitioned lung volumes: Ossification of the rib cage begins *in utero* and continues until around 25 years of age, thus the chest wall is more compliant in younger children resulting in a different balance between the outward chest recoil and inward lung recoil. In addition to the changes in the chest wall, younger children also have lower muscle strength which also impacts on the partitioned lung volumes. Since FRC reflects a balance between the elastic recoils of the lungs and chest wall, gravitational force and tension in the respiratory muscles, the functional implications of the growing (stiffening) chest wall, and developing respiratory muscles need to be considered when measuring and interpreting lung volumes in children.

1.3.2 Ethnic differences in lung function

The previous section highlighted the importance of considering growth and development when attempting to interpret lung function in children; however another important consideration is the impact of ethnic origin. Anthropometric differences have been observed in different ethnic groups, in particular the trunk: leg ratio has been shown to be higher in White European children in comparison to children of Black origin. Furthermore ethnic differences in lung function have been observed, with spirometric forced expiratory volumes and lung volumes being larger in White children compared to Black children[4-8] (Figure 1-13).

Figure 1-13: Predicted FEV_1 values for three ethnic groups.
Legend: Lower limit of normal (LLN) denoted by the dotted line.
Adapted from Quanjer *et al*, ERJ 2010[3]

The observed ethnic differences in lung function have been attributed to the anthropometric differences, although chest wall dimensions are also thought to contribute to these differences.[9] Several studies have reported lung function to vary from 10% to 25% between ethnic groups.[10] The ATS/ERS has suggested adjustment factors of ~12% for TLC, FEV_1 and FVC and ~7% for FRC when interpreting results from Black children.[2] Previous attempts however, to correct for ethnic differences in lung function have been shown to be over-simplistic,[7,8] and further work in interpreting ethnic differences is required.

1.4 Lung disease in children

Paediatric respiratory disease represents a major health issue, with asthma care and services alone costing the NHS almost £900 million per year (MRC-Asthma UK centre). In addition, there is evidence that much adult lung disease can be traced back to childhood.[11,12] Therefore the importance of identifying, monitoring and treating childhood lung disease cannot be over-estimated. This section will provide a broad overview of lung disease in children and the rationale for the lung diseases selected for further analysis in this thesis.

1.4.1 Overview of lung disease

In general, lung diseases can be categorised into those which cause an obstructive ventilatory defect, those which cause a restrictive ventilatory defect, and those which have a mixed obstructive/restrictive pattern. Some of these defects are illustrated in Figure 1-14, and lung function tests have been shown to offer an insight into defining these ventilatory defects.[2]

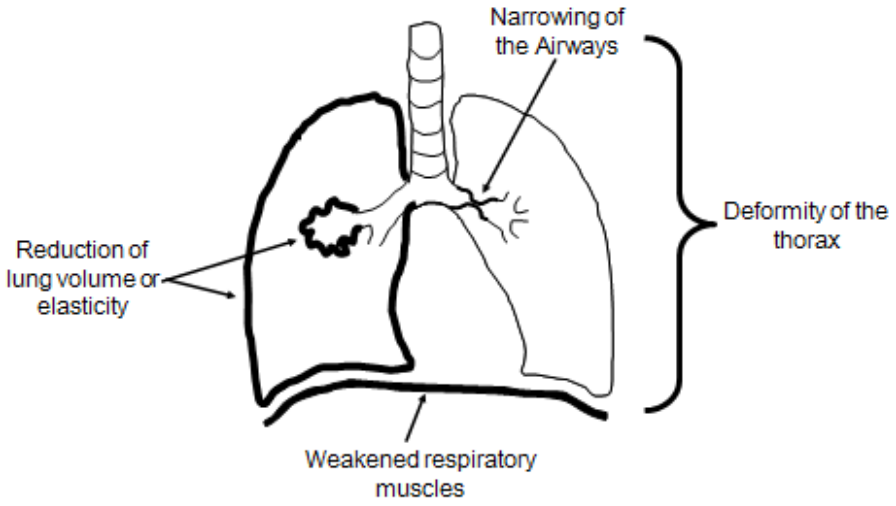

Figure 1-14: Schematic view of the differing mechanisms which cause lung disease.

An obstructive ventilatory defect is defined by a disproportionate reduction of maximal airflow from the lung in relation to the maximal volume (i.e. airflow limitation). In adults it is characterised by a reduced FEV_1/FVC ratio,[2] TLC may be normal, but RV is generally increased.

A restrictive ventilatory defect is defined by a restriction on the expansion of the lung, resulting in a lower lung volume (reduced FVC, reduced FEV_1, reduced TLC, however normal/increased FEV_1/FVC), increased work of breathing and inadequate ventilation.

A mixed obstructive/restrictive ventilatory defect is characterised by the coexistence of obstruction and restriction, and is defined by a reduced FEV_1/FVC and reduced TLC.

There are many paediatric conditions with significant respiratory symptoms and complications, some of which are listed in Table 1-1. Lung function tests (LFT's) offer a role in identifying obstructive or restrictive lung function abnormalities and monitoring respiratory status and response to intervention in these conditions.

Table 1-1: Common medical conditions that cause lung abnormalities in children.

	Description	Obstructive	Restrictive	Mixed
Asthma	Caused by a combination of genetic suspectability and environmental exposures. Symptoms include coughing, wheezing, chest-tightness and shortness of breath.[13]	✓		
Sickle cell disease (SCD)	Genetic blood disorder causing abnormal form of haemoglobin within the blood cells which then stick together causing obstructions within the blood vessels.[14]			✓
Cystic fibrosis (CF)	Genetic disease caused by a mutation in the gene for the protein cystic fibrosis transmembrane conductance regulator (CFTR), which is required to regulate transfer of salt at the surface membranes of the lungs and pancreas. The inability to excrete salt from the lungs results in a build-up of mucus, which contributes to airway obstruction, frequent infections and scarring of the lung.[15]	✓		
Bronchopulmonary dysplasia (BPD)	A chronic lung disorder that is common among preterm children with low birth weights and received prolonged mechanical ventilation. It is characterised by inflammation and scarring of the lungs and long-term respiratory morbidity has been described.[16]			✓
Congenital lung abnormalities	An incomplete development of the lungs *in utero* will result in an abnormally low number or size of alveoli[17]		✓	
Chest deformities	e.g. Scoliosis: A condition in which the spine is curved and can restrict the movement of the thorax[18]		✓	
Neuromuscular weakness	Weakness in the respiratory muscles prevents adequate expansion of the lungs[19]		✓	
Interstitial lung diseases (ILD)	ILD are a group of diseases that inflame or scar the lungs. Resulting in pulmonary fibrosis which limits the expansion of the lungs[20]		✓	

1.4.2 Rationale for patient group under investigation: Sickle Cell Disease

Sickle Cell Disease (SCD) is an inherited blood disorder in which the red blood cells contain an abnormal form of haemoglobin (Hb), causing them to become misshaped (sickle shaped). The sickle-shaped cells stick together and to the lining of the blood vessels and cause obstructions (infarcts) in the blood stream. SCD is one of the most prevalent genetic diseases with around 1 in 200 Afro-Caribbean children in the UK and 1 in 400 Afro-Caribbean children in the USA born with SCD.[21] It is associated with many pulmonary complications,[14] as well as having a strong association with asthma.[22] Acute complications include pneumonia, thromboembolism, fat embolism and acute chest syndrome (ACS), whilst chronic complications include sickle cell chronic lung disease with death usually occurring within seven years of diagnosis.[23] Thus SCD can result in significant respiratory morbidity,[14] with studies to suggest that SCD progresses from an obstructive lung defect in childhood[24] to a predominantly restrictive defect in adulthood.[25] Lung function measurements in children with SCD could therefore potentially play an important role in the clinical management of children with SCD, however interpretation can be challenging without ethnic specific lung function reference equations. Consequently, a group of healthy control children from the same ethnic background will also be investigated as part of this thesis.

1.5 Lung Function Tests

Lung Function Tests (LFT's) are used in both clinical care and research projects and, when applied in combination with a clinical history and other diagnostic tests, can be used:

- to confirm/exclude the presence of abnormality/disease
- as a guide to assess disease severity/progression
- to distinguish between obstructive and restrictive defects
- to monitor the course of disease or the effects of treatment

An accurate LFT may be the first and indispensable step in the process of clinical management. However, unlike with the situation with regards to drugs, which undergo rigorous testing and are registered with the Food and Drug Association (FDA), there are no formal requirements that lung function tests need to meet in order to be accepted or retained as a routine part of healthcare. Hence careful evaluations of LFT's are required before attempting to use them either in clinical management or as objective outcomes in research, and the following criteria should be considered:

- Patient group (i.e. age, suspected underlying pathology, ability to follow instructions (co-ordination and co-operation)),
- Equipment (i.e. whether there is commercialised equipment available and the ease of set-up and maintenance of this equipment),
- Environment to be used in (i.e. specialised laboratory, ward, outpatients, school or other "field" environment),
- Methods of data collection and analysis (i.e. whether tests are standardised for use in that particular patient group, and if adhered to equipment specifications),
- Within-subject variability for the age/patient group being investigated, and
- Availability of appropriate reference data.

In addition, financial implications (e.g. the cost of equipment, consumables, time, expertise, and the number of technicians required) and manufacturer's support (software guidance/upgrade and data handling) can have important implications.

Although LFT's are thought to be a valuable adjunct to the clinical management of respiratory disease, appropriate methods and interpretation are essential to optimise their use. Assessments of data quality control, knowledge of the normal variability of the LFT, and appropriate use of reference data should be evaluated prior to making any clinical interpretation. These three broad categories are described briefly here, and further discussed in relation to the LFT's under investigation in sections 1.6 to 1.9.

1.5.1 Application and Interpretation of lung function tests

1.5.1.1 Quality control

Validated equipment and standardised methods are the first steps to ensuring accurate assessments are achieved. LFT's however, may involve recording tidal breathing patterns which are inherently difficult to standardise, or maximal expiratory manoeuvres which are effort dependent. Thus the very nature of LFT's means that considerable variation can occur due to a number of issues (e.g. artefact, irregular breathing pattern, poor co-operation, etc.). All lung function results therefore need to undergo some level of quality control (QC) to determine if they are technically satisfactory. These QC assessments give an indication of how reliable data are, such that, if the data do not meet the QC criteria, less confidence can be placed on the result. For some LFT's there are well defined QC criteria, whereas others may need more attention towards standardisation of quality control, particularly in children.

1.5.1.2 Repeatability

After assessing the quality of data and determining the test is an "acceptable" measure, the within-test variability of the LFT should be assessed. All LFT's are repeated a number of times and, depending on the type of test, the "best" (highest, mean or median) of a number of trials are reported. An ATS/ERS task force defined **Repeatability** as "the closeness of agreement between the results of successive measurements of the same item carried out subject to all of the following conditions: same method, same observer, same instrument, same location, same condition of use, and repeated over a short space of time"; Whereas the term **reproducibility** should be used only if conditions have changed.[26] Variability may be expressed as within-subject standard deviation or the coefficient of variation (CV) which is the standard deviation expressed as a percentage of the mean, and can be assessed in several ways (e.g. within-test repeatability, between-test repeatability, or long term reproducibility) and is dependent on the LFT applied and the age group assessed.

Within-test, within-subject repeatability reflects the consistency of the subject "effort", the stability of intrinsic biological factors (i.e. the subject's breathing pattern) and the precision of the LFT device used. It is used to assess flow-limitation and to ensure that maximum effort has been achieved, or that a stable resting lung volume (FRC) has been established. For some measures the within test repeatability is defined within the guidelines of an "acceptable test session" however others may be less well defined.

Between-test repeatability (i.e. short term repeatability within a 15 minute period) is also important, as this allows the determination of whether an apparent change over time in response to an intervention is clinically significant. Between-test repeatability may be influenced by disease and biological variability in lung function, the stability of the LFT device and the technical consistency of the subject. If the between test repeatability is very low, subtle changes from this baseline measure can be detected. However, if the between test repeatability is large, then any "clinically significant change" must be something over and above that seen in the between test repeatability. The threshold for a significant bronchodilator response (BDR) can be determined by establishing the within-subject between-test repeatability, whereby a response greater than twice the average baseline CV can be considered positive.[1]

Long-term reproducibility (i.e. variability over several weeks or months) can be also be assessed, however this is beyond the remit of this thesis.

1.5.1.3 Compare to reference data

Once a LFT has undergone QC and the repeatability of the measure has been confirmed, the result should be compared to a known reference to determine if it is a "normal" or "atypical" result. In the simplest terms the subject can act as their own reference to determine if there has been a significant change from baseline (e.g. the effect of an intervention, or change over time). This method, however, does not give an indication of whether the child differs from the normal population, and is further limited in growing children as it does not take into account the effect of growth and development. Recruitment of a prospective control group with similar characteristics as the index group allows the determination of group differences; however a more comprehensive approach is to compare results with published normative reference data.

The principles behind normative reference data are based upon the theory that a summary measure of values obtained from "normal" individuals will represent the range of values expected in a healthy population.[27] Thus the reference range selected to interpret results should be derived from a similar "normal" population from which the test subjects come from, using the same equipment and methodologies as those applied.[28] It is not feasible for every individual lung function laboratory to generate their own reference ranges as the sample size needs to be large enough to ensure that the extreme limits of "normal" can be estimated with reasonable precision. Furthermore, despite the ATS/ERS guidelines which recommend validating reference equations to

ensure they match the healthy population,[26] this is not feasible in the majority of lung function laboratories. Many investigators simply rely on software defaults within their lung function equipment, and a recent survey of lung function laboratories found that up to 25% of laboratories are not aware which reference equations were used in their centre and only one third had selected equations that are applicable to their own population.[29,30] Inevitably there will be differences between the predicted values in different studies, and the choice of reference equation has been shown to have important implications on interpretation.[31]

Alongside the challenges faced with selecting appropriate reference data, expressing results in a meaningful way has its own challenges. Interpretation of reference data are based on the assumption that the values observed in the population are normally distributed (i.e. fit the Gaussian distribution) such that 95% of the values will fall within approximately two standard deviations (SD) of the mean. Results can then be expressed as Z scores (or SD scores) which are calculated as:
[observed-predicted)/(SD-predicted)].
Z scores quantify how far from the mean an individual observation is. Depending on outcome, lower or upper limits of normality (LLN or ULN) may be defined either as those encompassing 90% of the healthy population, in which case the LLN and ULN are based on the 5th and 95th centiles (i.e. ±1.64 SD) or alternatively encompassing 95% of the population, whereby the LLN and ULN represent the 2.5th and 97.5th centiles (±1.96 SD) respectively. Values that fall outside this range do not necessarily indicate abnormality, rather that further investigation (i.e. repeat or additional assessments in combination with clinical history) is warranted.

Percent predicted is another method of expressing lung function results. This is calculated as: **[(observed/predicted)*100]**
Percent predicted is a common outcome which is easily understood by both clinicians and patients. The limitation of percent predicted is that it does not take into consideration the variability of values around the mean, and the LLN is based on a constant proportion of the mean. The implications of this are that in younger children and the elderly in whom greater variability around the mean is observed, fixed percent predicted cut-offs may over-estimate abnormality.[32] The debate on expressing predicted values has been on-going for some time,[33] however, as long as the SD of the population is available, both methods can be utilised.

There is a wide variety of LFT's available, however this thesis will be limited to the evaluation of four commercially available LFT's that are used currently for both clinical and research purposes: Impulse Oscillation (IOS); Specific Airways Resistance (sR_{aw}); spirometry and plethysmographic lung volumes (literature as of inception of thesis: 2008).

1.6 Oscillometry

Oscillometry is an effort independent measurement which gives an assessment of total respiratory resistance.

1.6.1 Principles of oscillometry

The fundamental assumption of oscillometry is that respiratory mechanics can be measured by superimposing external pressure oscillations on the respiratory system during resting breathing, and analysing the resulting pressure and flow response. The spectral ratio of the amplitude of the pressure wave signal to the resulting flow signal constitutes the impedance of the total respiratory system (Z_{rs}), through which the total resistance (R_{rs}) and reactance (X_{rs}) of the total respiratory system is calculated. Z_{rs} can be conceived as a generalisation of R_{rs} since it embodies the in-phase and out-of-phase relationships between pressure (P) and flow (V').

R_{rs} (the in-phase portion) describes the dissipative mechanical properties of the respiratory system, and X_{rs} (the out-of-phase (imaginary) portion) is related to the energy storage capacity and is thus determined jointly by the elastic properties dominant at low oscillation frequencies and the inertive properties which become progressively more important with increasing frequency (see Figure 1-5, section 0).

Depending on the condition of the lungs, a characteristic ratio between impulse pressure and flow can be recorded that is dependent on the frequency of oscillation applied to the lung. If a high frequency is applied, the amplitude of such a movement remains predominantly within the upper part of the airways, and will not penetrate down to the peripheral part of the lungs. Therefore the reflected waves at high frequency (>20Hz) only carry information about the upper airways (central airway resistance (R_{20})). In contrast, low frequencies penetrate down to the peripheral parts of the lungs, and the reflected waves carry information from both the peripheral and the central parts of the lungs. Thus, the low frequencies (5Hz) carry the sum of both peripheral as well as central airway resistance and the difference between values at 5Hz and 20Hz (R_5 and R_{20}) represents the peripheral airway resistance (Figure 1-15).

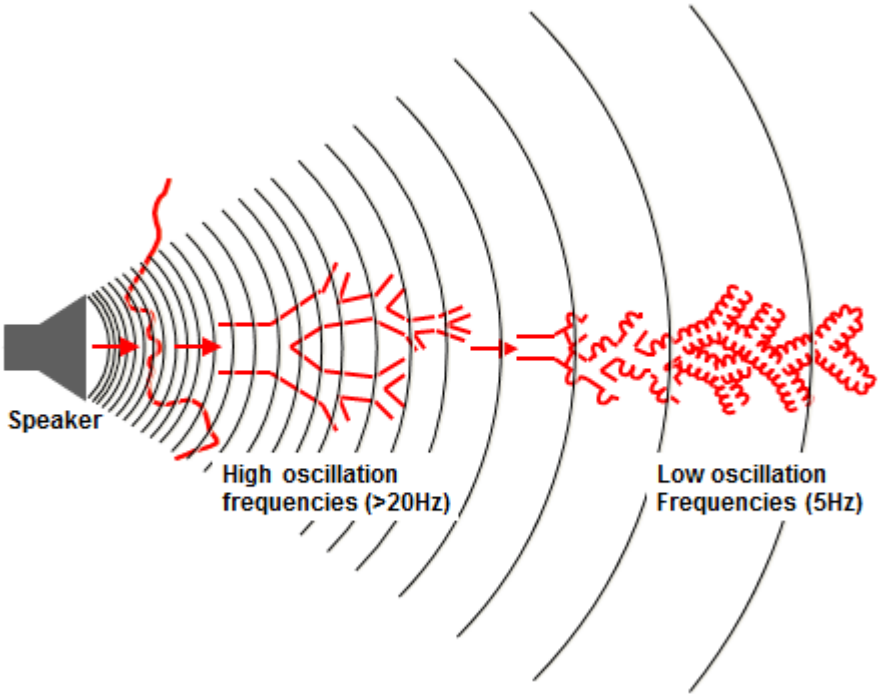

Figure 1-15: Multiple frequencies are applied to the respiratory system.
High frequencies remain in the upper airways, whereas low frequencies penetrate the peripheral airways.

1.6.2 Equipment specification for the oscillation technique

The advantages of oscillometry are that it is a non-invasive, versatile method which demands minimal patient cooperation, and is well supported theoretically. The full protocol and equipment set up can be found in the methods (section 2.6.4). The equipment is commercially available (costing ~£10k), requires minimal calibration and is portable (can be wheeled from one location to another). Historically there have been several methods of measuring oscillation mechanics, originating in 1956 by DuBois *et al*, with fixed frequency or monotonic / monofrequency measurements.[34] This is the simplest measurement in which one frequency, which usually ranges from between 2-4Hz to 30-35Hz, is applied at an individual time. Whilst the information obtained from this technique is valid, it is a time-consuming technique and there is no assessment of the frequency response of impedance (Z_{rs}). It cannot therefore differentiate between resistive and visco-elastic elements of the respiratory system.

The limitations associated with mono-frequency measurements were overcome as computer software improved, and the development of Fast Fourier Transformation (FFT). FFT allowed multiple frequencies to be analysed at once, allowing better definition of the signals from the out-of-phase portion of the respiratory cycle.[35] The continuous / multi-frequency harmonic oscillation measurement utilises computer driven forced pressure oscillations, including a wide range of frequencies applied in a

single burst, commonly between 2 and 48Hz. This is referred to as Pseudo Random Noise (PRN) Forced Oscillation Test (FOT), and is a method in common use.[36,37] PRN allows Z_{rs} to be estimated simultaneously at a number of frequencies, thus providing insight into the frequency-dependent mechanics of the respiratory system.

Time discrete, Impulse Oscillation is a step further on from PRN and works by superimposing many frequencies, and adding them together to generate a so-called "rectangular pulse". "Impulse Oscillometry" (IOS) is performed by applying this rectangular pulse signal to the airways with a pressure step wave every 200 milliseconds through a loudspeaker / pulse generator to the airway opening via a mouthpiece. The superimposed pressure oscillations during normal spontaneous breathing are composed of several frequencies allowing assessment (by Fast Fourier analyser) of R_{rs} and X_{rs} at several frequencies simultaneously. Since all frequencies are applied in just one rectangular pulse, the measurement time is reduced whilst maintaining a very low noise to signal ratio. Furthermore, continuous curves are generated rather than values at discrete frequencies.[38]

Different oscillation techniques and instrumentation vary significantly, with most published data prior to the mid-1990s being collected from "home-made" devices. Currently commercially available equipment includes the SensorMedics ROS, the 12M:Chess Medical which utilises PRN, and Jaeger IOS, which utilises rectangular wave "pulses". The limited number of studies comparing the accuracy of IOS to FOT generally suggests similar, but not identical measures of R_{rs} and X_{rs}.[39] Hellinckx *et al* compared the Jaeger IOS with the SensorMedics FOT system in a random order in 49 subjects (age range 8 to 70 years) with a variety of respiratory diseases. The wide range of resistances made a reliable comparison and found that the two techniques to be closely correlated ($r^2 = 0.83$), with R_{rs} values measured by IOS being slightly higher than those measured with FOT at all frequencies, whereas X_{rs} tended to be smaller with IOS.[40] Similar results were found in a pilot study of adults with COPD.[38]

1.6.3 Application and interpretation of the oscillation technique

1.6.3.1 Quality Control for IOS

As with all LFT's, IOS QC criteria can be checked both during and after the measurement. During the measurement the operator must coax the subject to adopt a quiet, relaxed breathing pattern free from artefact such as swallowing, coughing, chewing, talking and tongue movement which can cause airflow leak. It is the operator's responsibility to optimise data quality by observing the real-time volume/time display to identify data corruption (i.e. airflow leak) as described in the FOT recommendations by Oostveen *et al*.[39] If artefact/airflow leak is detected, the decision on whether to exclude the entire measurement as suggested by some investigators,[36] or to select a time segment free from artefact which meets a minimum time specification (e.g. 20 seconds)[41] needs to be made. Guidance for identifying artefacts, and selecting "acceptable" segments has been provided by Goldman *et al*,[38] but as yet there is no international consensus on quality control. Selecting "acceptable" sections on the results can be subjective since there is no method of quantifying airflow leak; this subjective analysis could therefore lead to increased inter-observer variability.[42]

Standardised guidelines for IOS are limited. In 2003 some guidelines for FOT were published by Oostveen *et al*[39], however the IOS and FOT are not directly comparable and discrepancies in QC have already been highlighted. Further work on standardising QC for IOS in children is required.

1.6.3.2 Repeatability in IOS

The coefficient of variation (CV) has been recommended to be used as the main index of the reliability and repeatability of Z_{rs} data[39] and has been described in a limited number of studies with conflicting results: In one study the repeatability of replicate IOS measures at a single sitting, and serial data over three consecutive days, in adolescent subjects (n=24, mean age (SD) 12.7 (1.8) years) with well-controlled asthma found that in contrast to spirometry, significant day-to-day differences occurred.[43] The within-day CV was substantially less than day-to-day variability, such that day-to-day changes in these parameters for each individual were more than twice the within-day CV for the same individual. They concluded that 60% of the adolescent asthmatic subjects tested demonstrated day-to-day variability in R_5 and AX that were in the order of 15-35%, variations were thought to reflect real physiological changes rather than random differences.[43] Another study evaluated IOS outcomes (R_5, R_{20} and

X_5) in triplicate at baseline and after a placebo in 33 asthmatic preschool children (aged 3 to 6 years) and found the baseline CV for R_5 to be 4.1%.[44]

In summary, the short-term CV (between-test repeatability) of R_{rs} derived by various oscillation techniques has been reported to range between 5-15% which is comparable, but always slightly larger than the variability of other lung function methods.[39,45] The within-test and between-test CV in oscillation may be related to the technique employed (e.g. CV appears to be lower for FOT than IOS) and the methods of analysis (CV can be reduced when a reliability index is applied) and may also be influenced by the parameters investigated (i.e. the lowest oscillation frequencies have the greatest variability because this is closer to the natural harmonics of tidal breathing), and the subjects tested (the examples above investigated lung disease, but the variability may be different in health or in different disease states). These factors need to be taken into account when determining thresholds for significant change in lung function or the response to an intervention and further investigation is required.

1.6.3.3 IOS and the Bronchodilator Response

Several studies have demonstrated a BDR with IOS in healthy children: Houghton *et al* demonstrated a mean reduction in R_5 of 7.9% in 12 healthy children, but a significantly larger reduction of 11.3% in 12 asthmatics.[46] Nielsen *et al* had a larger group (37 healthy controls and 55 asthmatic subjects) and also found that healthy children showed significant improvements post BD compared to placebo (5.1%, and 9.5% reduction in R5 post placebo and BD respectively), although the BDR in asthmatic children was significantly greater (17.7% reduction).[47] A larger BDR was demonstrated in 4 year old children in the Childhood Asthma Prevention Study where the BDR in health and asthma for R5 was 17% vs. 27% respectively.[48] Statistically significant BDR in both healthy children and those with lung disease measured with PRN FOT have also been reported.[37]

Given that BDR has been observed in health, there are discrepancies in what constitutes a significant BDR and which parameter should be used to determine this. Suggestions of “meaningful” reductions in R_5 have ranged from 20-25%,[47,48] up to 40%,[49] whilst expected changes in R_{10} are a little lower, ranging from 15-20%[48] up to 30%.[50] In contrast, changes of up to 65% have been recommended for AX,[37] which may be due to baseline variability or low absolute numbers. There is an urgent need for consensus on the threshold for a significant BDR for each outcome (specific to equipment) to be established.

1.6.3.4 Oscillometry in health

Studies on healthy children are essential to characterise the pattern of oscillation results, establish the normal variability expected within and between tests, develop reference equations specific to children, and to determine significance of therapeutic interventions. Several studies have demonstrated frequency dependence of resistance (Fdr) of resistance in young children: Stanescu *et al*, 1979, measured R_{rs} in 130 subjects aged 3 to14 years using forced oscillations between 4 and 9 Hz and found Fdr (R_4-R_9) in children at all ages, which decreased with increasing height (r = -0.50).[51] They found that during growth, peripheral resistance decreased and R_{rs} become less Fdr, such that by the age of 15 to 16 years R_{rs} was relatively independent of frequency, with no statistical difference between boys and girls.[51] In 1987, Clement *et al* extended the age range (4 to 20 years) and the number of healthy subjects (n=403) and demonstrated a relationship between R_{rs} and X_{rs} with age, height, and weight which differed between the sexes.[52] During growth, Fdr of R_{rs} decreased while X_{rs} increased, and the magnitude of the Fdr was related to age with those <10 years demonstrating a decrease in R4-R16Hz of about 10%, this Fdr disappeared at about 13.5 years, and adult values for R_{rs} and X_{rs} were attained by around 15 years of age in girls and 18 years in boys.[52]

Subsequently Cuijpers *et al*, (1993) measured FOT in 371 healthy children, aged 5 to 12 years and also demonstrated a reduction in R_{rs} with increasing height, and the negative Fdr decreased with growth, however the sex differences reversed once children reached 140 cm in height, with girls having higher R8 <140 cm, and boys having greater R_8 above it.[53] In summary, Fdr of R_{rs} occurs in normal healthy children up to the age of about 13 years, and may be more pronounced in boys; this Fdr of R_{rs} is likely to represent developmental changes rather than implicate pulmonary pathology, and should be considered when interpreting results.

1.6.3.5 Reference Data in IOS

The ERS taskforce on “The Forced Oscillation in Clinical Practice” in 2003 reported reference FOT data in adults to be virtually stable and frequency-independent, with mean R_{rs} in females being slightly higher than males (0.31 $kPa \cdot L^{-1}.s$ vs. 0.25 $kPa \cdot L^{-1}.s$). The same review reported on 11 reference equations on FOT in children published between 1979 and 1993 which all revealed a negative correlation between R_{rs} and height, a Fdr of resistance which became less pronounced with increasing height, and a high resonant frequency (F_{res}) in small children (up to 20Hz), which decreased as X_{rs} became less negative with growth. The authors concluded that “despite lack of

standardisation in measuring procedures and equipment, reasonably good agreement amongst the reference equations have been observed."[39]

More recently the 2007 ATS/ERS Official Statement on Pulmonary Function Testing in Preschool Children highlighted eleven reference equations for FOT in children,[1] seven of which were mentioned in the 2003 report.[39] The equations were published between 1979 and 2002, and showed similar agreement with R_{rs} decreasing with height (Figure 1-16).[1] The clear outlier (highest R_{rs} values) comes from the Klug and Bisgaard dataset. These investigators used modified facemasks which potentially could have introduced additional upper airway compliance or nasal breathing.[54] Only three of the equations presented in the official statement had included multiple frequencies that were taken from more than one measurement,[50,55,56] and none of these equations related to measurements obtained using the Jaeger IOS system. Although FOT and IOS are similar, the results obtained using these techniques should not be regarded as interchangeable.[40]

Figure 1-16: Rrs at different frequencies according to published reference data.
Legend: The lines represent the following published reference equations (numbers represent cited ref in original publication: 174=Klug and Bisggard,[54] 190=Solyman *et al*,[57] 186=Mamberg *et al*,[50] 189=Hordvik *et al*,[55] 188=Lebecque *et al*,[58] 193=Hellinckx *et al*,[49] 194=Stanescu *et al*,[51] 295=Hantos *et al*,[59] 195=Ducharme *et al*,[60] and 5=Mazurek *et al*.[61]

In addition to the ATS/ERS preschool statement, a limited number of independent IOS-specific reference equations for children have been produced: In 2005, Frei *et al* generated reference equations from 222 White North American children aged 3 to 10 years (height 100-150cm),[62] while in 2006, Dencker *et al* collated IOS data from two populations: 109 Finnish pre-school children aged 2 to 7 years, and 251 Swedish children aged 7 to11 years and calculated reference equations from all 360 children (90-160cm).[41] Ethnic specific reference equations have also been developed for Chinese children[63] and Iranian children,[64] while the largest collection of IOS healthy data to date comes from a Polish population of 626 children aged 3 to 19 years.[65] All 5 studies reported similar trends in R_{rs} and X_{rs}.

In summary, a variety of reference data are available for FOT and IOS, however reference equations developed for FOT may not be appropriate for IOS. Similarly reference equations collected in White subjects may not be appropriate for African American and Asian subjects, where ethnic differences in lung function have been described.[66] There is an urgent need for age, sex and ethnic specific reference data for each type of commercial equipment along with appropriate guidelines for their use.

1.6.3.6 Oscillometry in lung disease

Resistance measurements have shown higher mean values in populations of asthmatic children compared to healthy young children.[47] However, wide inter-subject variability of baseline measures limit the extent to which baseline abnormalities can be detected and there is little consensus on which threshold should be used to define an abnormal value. Despite this, several studies have successfully utilised oscillation techniques in conjunction with a bronchial challenge to assess airflow obstruction in children[45,67,68] and adults.[69-71] These techniques have also proved useful in the assessment of BDR.[37,49,72,73]

Several studies have demonstrated lung function abnormalities, and in particular asthma-like characteristics in SCD,[22,74,75] however only one study has utilised IOS (as a secondary) outcome: Santoli *et al* studied lung function and acute chest syndrome in 49 children with SCD, and demonstrated an increased R_{rs} that was associated with reduced expiratory flows and an increase in the number of ACS episodes.[76]

In summary, IOS has proved to be a potentially useful outcome measure in the assessment of airflow obstruction and asthma, however there has been limited use of IOS in the investigation of children with SCD.

1.7 Specific airways resistance (sR_{aw})

Lung function techniques that can be applied during tidal breathing are particularly pertinent in young children where active cooperation and understanding may be reduced.[1] Plethysmographic Specific Airways Resistance (sR_{aw}) is a measurement of airflow resistance, corrected for lung volume and can be used to identify airflow obstruction in subjects who are unable to perform a maximal forced expiratory manoeuvre.[2] It is measured during tidal breathing when the relationship between simultaneous measurements of airflow and the change in plethysmographic pressure is assessed without the need for any special breathing manoeuvres against an airway occlusion.[77] It is therefore ideally suited for young children.[78]

1.7.1 Principles of sR_{aw}

sR_{aw} is the product of Functional Residual Capacity (FRC) and Airways Resistance (R_{aw}). It can be calculated from the relationship of plethysmographic box pressure (P_{box}) to flow during spontaneous breathing. Derivation of sR_{aw} occurs as follows:

1) $\mathbf{R_{aw}} = (\Delta V_{box\_spontaneous} / \Delta flow) / (\Delta V_{box\_occlusion} / \Delta P_{mouth})$

2) FRC = $(\Delta V_{box\_occlusion} / \Delta P_{mouth}) \times (P_{amb} - P_{H20})$

Where

$\Delta V_{box\_spontaneous}$ = change in box volume during spontaneous breathing

$\Delta V_{box\_occlusion}$ = change in box volume during efforts against the airway occlusion (shutter)

$\triangle P_{mouth}$ = change in mouth (alveolar) pressure during efforts against the airway occlusion

P_{amb} = ambient pressure

P_{H2O} = water vapour pressure

Equations (1) and (2) are then combined:

3) $\mathbf{sR_{aw}} = \dfrac{\triangle V_{box\_spontaneous} / \triangle flow}{\triangle V_{box\_occlusion} / \triangle P_{mouth}} \cdot \dfrac{\triangle V_{box\_occlusion}}{\triangle P_{mouth}} \cdot (P_{amb}-P_{H2O})$

And simplified, thereby avoiding need for an airway occlusion with which to calibrate box pressure changes in terms of alveolar pressure changes.

4) $\mathbf{sR_{aw}} = \dfrac{\triangle V_{box\_spontaneous}}{\triangle flow} \cdot (P_{amb}-P_{H2O})$

In the simplest form, sR_{aw} can be derived from the tangent of the slope of box Pressure/Flow. Since R_{aw} has a strong inverse relationship to lung volume, sR_{aw} provides a relatively stable index with which to distinguish effects of disease from those of growth and development.[34,77]

1.7.2 Equipment specification for sR_{aw}

sR_{aw} can only be measured within the body plethysmograph which is a large "body box", approximately 160cm in height, 100cm wide and 78cm deep, and weighs around 40kg. It is therefore not portable and, although commercially available, it is more expensive than other lung function equipment (costing ~£30k) and therefore limited to specialised respiratory laboratories. Furthermore, there is a lack of consensus with regards to equipment, measurement conditions, data collection, analytical strategies and reference data. Consequently, reported values of sR_{aw} have been collected under a variety of differing measurement conditions: In the past, changes in temperature and humidity throughout the breathing cycle[79] have been compensated with either re-breathing bags or panting technique to achieve body temperature, pressure, and water vapour saturated (BTPS) conditions[80] whereas, in recent years, quiet tidal breathing with subsequent electronic compensation has been utilised. The application of a digital (electronic) 'thermal correction' factor during calculation of sR_{aw}[79] has been shown to produce systematically higher results than those collected under BTPS conditions,[81] thus even healthy subjects will appear to have abnormally elevated sR_{aw} if results are interpreted using BTPS-derived normative data.

In addition to the differing equipment specifications (NB: assumptions of plethysmography are described in section 1.8.1.1), measurements have been performed with modified facemasks[82] and mouthpieces,[83] with or without bacterial filters, which potentially vary the deadspace within the system. Each manufacturer has produced different software, and the relationship between plethysmographic (box) pressure and airflow can be analysed in a variety of ways, thus resulting in numerous different outcome measures for sR_{aw}, including: 'effective resistance' (sR_{eff}); 'total resistance' (sR_{tot}); 'peak resistance' (sR_{peak}) and resistance measured over a fixed range of flow (e.g. between 0-0.5 $L.s^{-1}$ i.e. $sR_{0.5}$). In children the most common outcomes are sR_{eff} and sR_{tot}, whilst measures of $sR_{0.5}$ have been discouraged in children due to potential age-related effects.[84] sR_{tot} is a simple outcome measured between points of maximum plethysmographic (box) pressure, however sR_{eff} may be a better reflection of airway mechanics as it is calculated from multiple points throughout the breathing cycle (the integration method)[6,85] (see Chapter 2, section 2.6.5.3 for further details).

1.7.3 Application and interpretation of sR_{aw}

1.7.3.1 Quality Control for sR_{aw}

The use of sR_{aw} as a valid outcome measure in clinical management has been limited by the lack of consensus with regard to equipment, measurement conditions, data collection, analytical strategies and reference data. Results may be influenced by:
a) the extent to which operator QC is used, either to exclude pressure-flow loops due to poor phasing/irregular breathing patterns, or manually adjust the automatically generated tangents for such loops;
b) the number of breaths per epoch or 'trial' and the number of trials used to summarise data, and
c) whether results are expressed as the median of all data[86] or the weighted mean of data selected after extensive quality control.[87]
There are no standardised guidelines for QC applicable to sR_{aw} measurements.

1.7.3.2 Repeatability in sR_{aw}

Within-subject SD and between-subject SD in healthy young children (aged 3 to 7 years) have been reported to range from 0.086 to 0.109 kPa·s and 0.19 to 0.20 kPa·s, respectively.[78] This equates to a within-subject coefficient of variation of 8-11%. Repeatability has been shown to be independent of age.[54]

1.7.3.3 sR_{aw} in health

Dab and Alexander first described the technique in 1976[77] and subsequently determined no significant change in sR_{aw} with body size for normal children between 3 and 16 years of age.[84] In 2001, Manzke *et al* measured 187 girls and 213 boys aged 6 to 16 years and also found that sR_{aw} remained constant throughout childhood and adolescence, however they found a statistically significant difference between boys and girls and therefore recommended separate reference values for each sex.[6] More recently, Bisgaard *et al* measured 121 healthy 2 to 7 year olds (61 boys) with approximately 20 children in each age year and found sR_{aw} to be independent of height and gender.[78] These studies suggest that sR_{aw} remains fairly constant throughout childhood, with possibly some sex differences in older children.

1.7.3.4 Reference Data for sR_{aw}

sR_{aw} is the product of Functional Residual Capacity (FRC) and Airways Resistance (R_{aw}) (section 1.7.1). Since R_{aw} has a strong inverse relationship to lung volume,[77] sR_{aw} provides a relatively stable index with which to distinguish effects of disease from those of growth and development. sR_{aw} reference data to date, therefore quotes a

single reference value: Bisgaard *et al*, suggests a normal mean (SD) value of 1.3 (0.1) kPa·s for both sexes and all ages (2 to 7 years),[78] whilst the Carefusion software (which does not include data by Bisgaard *et al*) simply has a default value called "Jaeger-kids" for children aged 4 to 18 years, and "Jaeger" for those >18 years. These equations are based on unpublished data collected under BTPS conditions over 30 years ago[80] which are known to be systematically lower than those collected under electronic conditions.[81] The 'Jaeger-kids' predicted values for both sR_{eff} and sR_{tot} are 0.51 kPa·s for girls and 0.53 kPa·s for boys. There after follows by a sudden (and physiologically implausible) *increase* in predicted values to 0.96 kPa·s for females and 1.18 kPa·s for males from 18 years of age onwards. These default equations significantly under-estimate the actual values observed in healthy children, and if results were interpreted with these equations, a serious over-estimation of the degree of airway obstruction in children with lung disease would occur.

There is some evidence to suggest that there are age and/or gender differences in sR_{aw} in young children,[6,88] and updated reference data are urgently required.

1.7.3.5 sR_{aw} in lung disease

Researchers at Copenhagen University, Denmark, have investigated the use of sR_{aw} in children with asthma/wheeze and demonstrated that sR_{aw} is significantly increased compared to healthy children in groups of 2 to 6 year old asthmatic children.[47,89-92] The same investigators evaluated the association between anti-asthma medication and sR_{aw} and concluded that sR_{aw} is a useful outcome measure to distinguish controlled from uncontrolled asthma.[89] Lowe *et al* also assessed sR_{aw} in 463 three year olds and repeated these measurements in the same children as well as some additional children at 6 years (n=690). In this group of pre-schoolers, they found that sR_{aw} could differentiate between persistent and transient wheezers and between wheezing and non-wheezing children, and that the deficit in lung function was considerably greater in persistent wheezers.[60-64]

In summary, measurements of sR_{aw} have proved to be a feasible and useful outcome measure in clinical research studies of preschool children with cystic fibrosis and wheezing disorders, but their usefulness in Sickle Cell Disease has yet to be determined.

1.8 Plethysmographic Lung Volumes

Measurements of lung volumes are the gold standard for identifying restrictive lung defects[2] and although not mandatory to identify obstructive defects, they may help to identify underlying pathophysiology. Reduced RV, FRC, TLC and RV/TLC ratio are commonly seen with restrictive disorders, whereas an increase in RV, FRC, TLC, and/or increased RV/TLC ratio denotes air trapping or hyperinflation associated with an obstructive pattern.

1.8.1 Principles of measuring lung volume assessments

Plethysmographic measurements are based on Boyle's Law which states that: under isothermal conditions, when a constant mass of gas is compressed or decompressed, the changes in gas volume and pressure are inversely proportional, such that the product of volume and pressure at any given moment is constant.

The subject is seated inside the plethysmograph and asked to breathe normally. Once stable tidal breathing has been established, an occlusion at the airway opening is made by closing a shutter to temporarily stop airflow (resulting in a fixed mass of gas in the lungs). Inspiratory efforts against the occlusion cause an increase in the volume in the lung and a decrease in alveolar pressure (P_{alv}) which equilibrates throughout the respiratory system (providing no airflow occurs) such that P_{alv} can be measured at the airway opening (measured as: change in mouth pressure: (ΔP_{mouth})). Simultaneously, the expansion of the thorax compresses the gas in the plethysmograph resulting in a decrease in box volume (ΔVbox) and an increase in box pressure during an inspiratory effort and the opposite during an expiratory effort.

FRC can be then be calculated: $(\Delta \mathbf{V_{box}} / \Delta \mathbf{P_{mouth}}) \times (\mathbf{P_{amb} - P_{H2O}})$

Where: ΔV_{box}: is the change in lung volume (calculated from change in box volume)

ΔP_{mouth}: is the change in alveolar pressure (calculated from P_{mouth} changes)

P_{amb} : Ambient pressure.

Maximal inspiratory and expiratory manoeuvres post release or airway occlusion allow the calculation of absolute lung volumes whereby

RV = FRC-ERV

TLC = RV + VC

The large volume of the plethysmograph box undergoes very small pressure changes during compression and decompression of TGV, thus the plethysmographic pressure

transducer must be very sensitive and stable. The plethysmographic transducer is calibrated in terms of changes in TGV by injecting and withdrawing a fixed volume of air (generally 30 or 50 mL) in and out of the plethysmograph using a motor-driven syringe to simulate the changes in TGV that occur during decompression and compression of thoracic gas during inspiratory and expiratory phases of the respiratory cycle. After such calibration, the measured changes in plethysmographic gas pressure reflect the change in TGV due to decompression and compression of thoracic gas. Changes in calibrated plethysmographic gas pressure are recorded in terms of volume change.

Since calibration of the plethysmograph is normally carried out without a subject in the plethysmograph, the calibration must be corrected for the subjects body volume (i.e. body weight must be entered prior to testing).

1.8.1.1 Assumptions of plethysmography

During plethysmographic measurements the airway opening is briefly occluded to hold the lung at a constant volume (normally at end expiration, i.e. FRC). Respiratory efforts that compress and rarefy the thoracic volume are recorded. By relating changes in P_{alv} (reflected by pressure changes at the airway opening during periods of no airflow) to changes in thoracic gas alveolar volume (reciprocal to pressure changes in the plethysmograph), thoracic gas volume at the moment of the occlusion can be calculated. There are however a number of assumptions relating to this technique:

1.8.1.1.1 Pressure-volume changes in the body are isothermal

An underlying assumption of the technique is that the pressure–volume changes in the body are isothermal. During inspiration, air is warmed and humidified to body temperature and pressure under saturated conditions (BTPS), and air is cooled during expiration. It is assumed that any heat generated by warm, expired air is instantaneously lost to the surrounding tissue, such that changes of alveolar volume will occur under isothermal conditions. However, during rarefaction and compression of gas within the plethysmograph, heat may or may not be lost through the walls of the container (i.e. conditions within the plethysmograph are not isothermal). The plethysmograph must therefore be calibrated at an appropriate frequency to mimic respiratory efforts. The plethysmograph is also open to the atmosphere via a small leak with a mechanical time constant of between 5 and 25s. This controlled leak minimises slowly occurring pressure changes that are not related to respiratory manoeuvres, such as thermal drift (heating) caused by the presence of a subject breathing and body heat within a closed chamber.

1.8.1.1.2 Linear behaviour between changes in P_{mouth} and P_{alv}

The respiratory system comprises both elastic recoil forces and resistance forces, thus a pressure change in the respiratory system will take a finite time to come to equilibrium. This time constant (τ) of the respiratory system is a function of the elastance/compliance and resistance of the system and is the time taken for 63% of a step change to stabilise.

Defined as: **τ=1/Elastance * Resistance** Or: **τ=compliance * resistance.**

Stiff lungs (low compliance) with a low resistance will therefore have a short τ and empty and fill rapidly, whereas lungs with normal/high compliance and high airways resistance will have a long τ and will empty/fill more slowly. The main assumption of body plethysmography is that changes in P_{mouth} closely approximate changes in P_{alv} during respiratory efforts against an occlusion (i.e. there is equilibration), however in airflow obstruction (where there is a long τ) the P_{mouth} and P_{alv} may not have time to equilibrate, hence the change in P_{mouth} may potentially under-estimate the changes in P_{alv} in the presence of airways obstruction and over-estimate total thoracic gas volume.

1.8.1.1.3 Pressure-volume changes are limited to the volume of gas within the thorax

Another assumption of body plethysmography is that changes in body volume during respiratory efforts against the occlusion are essentially only those of TGV, and that abdominal gas is negligible. Guidelines therefore recommend avoiding consumption of fizzy drinks prior to plethysmographic measurements, since increased abdominal gas and/or pressure swings could be a potential source of error.[93] Significantly, plethysmographic FRC measures all the total gas volume (TGV) in the lungs at end expiration, including any gas trapped behind closed airways. This contrasts to gas washout or dilution techniques (e.g. nitrogen washout) which rely on gas mixing, and only measure communicating ventilated areas of the lungs and not gas trapped in poorly or non-ventilated areas.

1.8.1.1.4 Pressure changes applied to the lung are homogeneous within the pleural space

Finally, the principles of body plethysmography are based on the assumption that pressure changes applied to the lung are homogeneous within the pleural space. The significant chest distortion observed in some infants with respiratory disease may be associated with inhomogenous pleural pressure swings during airway occlusion. Similarly, in the presence of marked ventilation inhomogeneity, ΔP_{mouth} may not reflect mean changes in $P_{alv,}$ potentially resulting in either over- or under-estimation of FRC.

1.8.2 Equipment specifications for lung volume measurements

Guidelines for equipment specifications have been developed.[94] The body plethysmograph used for the measurement of lung volumes was identical to that used for the measurement of $sR_{aw.}$

1.8.3 Application and interpretation of plethysmographic lung volumes

1.8.3.1 Quality Control for Lung Volume assessments

Measurements of absolute and partitioned lung volumes are technically more challenging than other LFT's and are therefore limited to older children. International guidelines for their use have been developed.[94] Three to five measurements are recommended to achieve at least three FRC values that agree within 5% of the highest value. A technically acceptable FRC is described as "a series of almost superimposed straight lines separated by only a small thermal drift on the pressure–volume plot."[94] These guidelines are based on adults, and while there is no evidence to suggest these guidelines are not feasible in children, at present there are no guidelines specific to children.

1.8.3.2 Repeatability in Lung Volume assessments

Guidelines state that within-test repeatability should be within 5%.[94] The same guidelines suggest that between-test repeatability in healthy subjects should not differ significantly (i.e. no greater than 10% for FRC and TLC and 20% for RV) from previously established means for measurements on the same subject, however absolute volume differences are not defined, and this doesn't growth into account. A study by Halvorsen *et al* measured 35 healthy children with a mean (SD) age 10.6 (0.4) years and 46 healthy children aged 17.8 (1.2) years and found the within-test repeatability (maximum value – minimum value) for FRC, TLC and RV to be 0.16 L, 0.13 L and 0.14 L respectively. They found no significant difference in the variance between gender, age or asthma status.[95]

Plethysmographic lung volumes have been successfully used as a BDR outcome measure in asthmatic children,[96] however it is a technically demanding measurement and time-consuming and therefore infrequently used as an outcome measure for BDR.

1.8.3.3 Lung volumes in health

Lung volumes are related to body size, with standing height being the most important determinant.[94] In children and adolescents, lung growth appears to lag behind the increase in standing height during the growth spurt, and there is a shift in relationship

between the lung volume and height during adolescence. Quanjer *et al* collated lung volume data from 2,253 children aged 4 to 20 years and analysed the pattern of anthropometric growth and changes in lung volumes.[3] TLC was a function of sex, age and height, and they found TLC to be 8% larger in males compared to females. RV/TLC ratio fell by about 5% in childhood until the start of adolescence where it increased about 2% during puberty, then decreased to the adult levels. This curvilinear pattern of RV/TLC ratio from childhood to adulthood can be explained by differences in the development of airway properties, body dimensions, chest shape and respiratory muscle function during growth, such that lung volume (size and number of alveoli) increase more rapidly than airway calibre during childhood.[3] Ethnic differences in lung volumes were also noted in this study, although limited numbers and differing methodologies prevented the group from presenting reference data. Ethnic differences have also been investigated by Sylvester *et al*, who studied 80 healthy Black children with a median age of 9 years and demonstrated significantly lower lung volumes than those values predicted from White reference ranges,[7] however they did not make a direct comparison between Black and White healthy children. Further work investigating the link between ethnic differences in lung function and somatic growth is required.

1.8.3.4 Reference Data in Lung Volumes

Lung volume reference data in children are limited and ethnic differences in such data are not well defined.[2] In the USA, the Zapletal equations are the most commonly used reference data,[97] whereas in the UK, the British Thoracic Society recommend reference equations by Rosenthal *et al*.[98] Zapletal's equations were based on a very small White population of 86 boys and 87 girls aged 6 to 17 years using out-dated (pre-electronic thermal compensation) equipment. The Rosenthal equations were based on modern equipment and a larger sample size (772) and included the calculation of Z scores. There are two equations (one pre-puberty and one post-puberty) for each outcome and each sex. The impact of these somewhat arbitrary pubertal break points are discussed further in the spirometry section below. Discrepancies in these equations in healthy children have been demonstrated previously.[16,99] To date, no lung volume equations specifically for Black children have been published, and previous attempts to correct for ethnic differences in lung function have been shown to be over-simplistic.[7,8]

1.8.3.5 Lung volumes in lung disease

SCD is thought to progress from an obstructive lung defect in childhood[24] to a predominantly restrictive defect in adulthood.[25] Several groups have identified a possible association of asthma and airways obstruction and SCD and recognised the

potential use of lung volume assessments to identify obstructive lung disease in children with SCD.[24,25,100,101] A study of 64 children with SCD and 64 ethnic-matched controls, aged 5 to16 years, which investigated whether children with SCD have restrictive lung function abnormalities revealed that children with SCD have significantly reduced lung volumes compared with healthy controls. These results suggest that restrictive abnormalities may become more prominent with increasing age.[10] The same group studied 20 children with SCD who had suffered acute chest syndrome (ACS) episodes and 20 children with SCD without ACS episodes, and found RV to be elevated (and a reduction in FEV_1 and flows) in those children who had suffered ACS.[102] This suggests ACS episodes predispose children to increased airway obstruction, and provides more evidence regarding the clinical usefulness of assessing lung volumes in children with SCD.

In summary, plethysmographic lung volume assessments are potentially useful outcome measures for distinguishing between restrictive and obstructive lung disease.[2] A brief review of the application of lung volumes in SCD has been summarised here. In addition, lung volumes have been proven to be useful in the assessment of children with asthma,[103] CF[104] and BPD.[16]

1.9 Spirometry

Spirometry is the most common lung function test available with well established guidelines for both adults[105] and children.[1] It is an effort dependent test which requires maximal inspirations and forced expirations, thus full subject cooperation is required and the potential for fatigue is greater than when using effort-independent tests such IOS and sR_{aw}.

1.9.1 Principles of Spirometry

Spirometry is the measurement of volume and/or flow of air inhaled or exhaled. Whilst measures of tidal volume can be recorded with spirometry, measures of maximal inspiratory and expiratory flow-volume are most informative. Details of airflow limitation which can be detected with spirometry assessments have been presented in section 1.2.7.

1.9.2 Equipment specification for spirometry

Spirometry can be performed using commercially available equipment that is portable. This test can be applied in both the hospital environment and in the field. The ATS/ERS re-issued equipment specifications and standardised guidelines in 2005.[105]

1.9.3 Application and interpretation of spirometry data

Spirometry is invaluable as a screening test of general respiratory health. In patients with respiratory disorders, several factors that influence the mechanical properties of the lung give rise to the distinctive shape of the flow-volume curves. Hence, inspection of the curves can contribute to clinical diagnosis. Forced expiratory flow has been discussed previously (section 1.2.6). Spirometry measurements are a cardinal feature for the assessment of respiratory conditions such as asthma and SCD. A vast body of evidence supports the applications of spirometry.[105-108]

1.9.3.1 Quality Control in Spirometry

Quality control guidelines are well established in adults[105] and young children[1] and laboratory based measures remain the "gold-standard" in both clinical and research practice.[109] Published evidence of disparity between practice and hospital services and the quality of spirometry performed[110] has fuelled the debate regarding the appropriateness of such measurements outside specialised facilities, however, with appropriate training and QC, spirometry can be equally feasible in the field environment.[111]

Recent ATS/ERS guidelines recommend a six second exhalation and no change in volume (i.e <25mL) in the last 1s of the forced expiratory manoeuvre in subjects >10 years, with a three second duration stipulated for younger children.[105] However, previous ERS recommendations did not stipulate a forced expired time (FET) for children as it was appreciated that many healthy young children may empty their lungs in less than three seconds.[112] In the recent EPICure study, modifications to current guidelines were made.[111] Recommendations included that all spirometry software should allow visual QC which can be coded for QC criteria as “second line QC”, thus allowing a more objective over-read of paediatric data.[111] In addition, the same study recommended spirometry software to display critical outcome measures with respect to QC, such as time to reach Peak Expiratory Flow, back extrapolation volume (BEV) as both absolute and %FVC, the % and absolute difference between best and 2nd best manoeuvres, the end of test volume (EOTV) and the FET, all of which were potentially adaptable but have not yet been implemented. Despite spirometry being the most commonly applied lung function test in adults and children, there are still unanswered questions regarding appropriate QC guidelines, particularly with respect to children. The Global Lungs Initiative (www.lungfunction.org) are currently updating these guidelines.

1.9.3.2 Repeatability in Spirometry

Repeatability in spirometry has been thoroughly investigated. International guidelines for within-test and between-test repeatability have been defined as <0.15L in FVC and FEV_1 between best and second best efforts, with a slightly lower criteria of 0.10L if the FVC is <1.0L.[105] These criteria have been shown to relatively lenient in adults, where a review of 18,000 consecutive patients, aged 20 to 90 years revealed that 90% of subjects were able to reproduce FEV_1 to within 120 mL (i.e. within 6.1%), and FVC within 150 mL (5.3%).[113] Similarly, a review of 4000 children aged 9 to 18 years revealed that most met these criterion.[114] In addition, in the more recent EPICure study, where repeatability criteria was more liberal (highest FEV_1 and FVC values were within 10% of each other) to allow for the potential neurological deficits in the extremely pre-term children assessed, the vast majority of children (aged 10 to 11 years) met the repeatability guidelines.[111] Preschool guidelines state that adult criteria are not suitable for young children and suggest repeatability within 0.1L or 10%,[1] however it is important to note that since spirometers only have accuracy to ~100mL it is difficult to impose closer repeatability criteria with confidence.

1.9.3.3 Reference Data in Spirometry

A plethora of spirometry reference data in children are available[115,116] with the most comprehensive reference data to date being the All-Age equations.[117] These equations are based on 7209 measurements from White subjects aged 4 to 80 years and updates on these equations are in progress (www.lungfunction.org).

For Black children, the only reference data available are those by Wang *et al*.[5] These were created from the 6 cities study which included 989 Black children aged 6 to 18yrs who underwent a total of 6,324 annual examinations (NB. Equations derived from 1630 White children are also available for this study). The outcomes were regressed on a logarithm of height, and reference equations were created which were sex and age specific (i.e. a separate equation for each sex and age group). The equations were limited to children aged 6 to 18 years thus a change in reference data to NHANES III is required when progressing to adulthood.[107] To date there are no Black-specific equations available for children <6 years.

1.10 Other LFT's (not used)

The LFT's which have been selected for this thesis do not comprise all the LFT's available for use in children. Other LFT's include the Multiple Breath Washout technique (MBW),[118] Oesophageal manometry,[119] Resistance measured by the

interrupter technique (Rint),[1] Diffusion capacity with carbon monoxide (DL_{CO})[120] and measurements of exhaled nitric oxide.[121,122]

1.11 Rationale for tests under investigation

Table 1-2 summarises the LFT's under investigation for this thesis with respect to the interpretation criteria suggested.

IOS is a theoretically promising test that is effort independent and shown to distinguish between health and disease. Equipment is commercially available, relatively portable and requires minimal maintenance. However, the clinical usefulness of this test is limited due to poor interpretation guidelines. There are currently no standardised international guidelines on QC and analysis and equipment specifications for data handling are limited. In particular the relatively low repeatability of R_{rs} over days and weeks may limit its applicability to longer-term follow-ups.[123] Investigation of the methods and appropriate QC criteria may improve within-test repeatability and optimise the feasibility of this test. Other limitations which need investigation include the lack of defined cut-off points for a positive BDR and clinically significant thresholds for the determination of airflow obstruction. In addition, the reference data available needs to be evaluated with respect to the impact of ethnicity, and different patient groups.

sR_{aw} has been shown to be a useful research tool able to differentiate between health and disease, but methods and equipment are not standardised and its clinical usefulness is limited. The numerous different methods of data collection, outcome measures and QC techniques have resulted in wide variations in practice. Standardisation for this technique is urgently required, similarly reference data needs to be investigated, and software adaptations are required.

Plethysmographic lung volume measurements are the gold standard for identifying restrictive lung disease.[94] Guidelines for QC and repeatability appear to be adequately defined, but not verified in children. Reference data have been reported to be unreliable in White children, and no Black reference data are available. An investigation into reference data and appropriate interpretation in terms of identifying restriction/obstruction is warranted.

Spirometry is the most commonly applied lung function technique. Guidelines on QC are available and repeatability is well established. Reference equations for White children are available; however interpretation in Black children may be limited due to the lack of ethnic-specific equations across all ages.

Table 1-2: Summary of QC, repeatability and reference data available for each LFT.

	Quality Control	Repeatability	Reference Data
IOS	Limited guidelines[1,39,124] Not standardised	Poorly defined: Within-test repeatability varies from 5-15%.	3 equations available for White children.[41,62,65] None available for Black children.
sR_{aw}	Limited guidelines, Not standardised	Well defined, but only by a single group[78]	Predicted values have been defined[78] Impact of ethnicity unknown.
Lung Volumes	Well defined in adults[94] and extrapolated to children	Described in adults[94] and children.[125]	Recommendations for the USA[97] and the UK[98] are based on White children. No ethnic specific equations are available
Spirometry	Well defined in adults[105] and young children[1] but some modifications may be required[111]	Well defined in adults[105] and children[1,113]	Plethora of data,[116] most comprehensive limited to White subjects.[117] One Black-specific equation for children >6y.[5]

1.12 Summary of introduction

This introduction chapter has described the basic physiology of the lungs during growth and development and highlighted the impact of ethnicity on lung function. It discussed the clinical implications of common lung diseases such as asthma and SCD and the importance of lung function assessments in identifying and managing these lung diseases from an early age. Four LFT's were identified for investigation and were reviewed in light of the interpretation steps: quality control, repeatability and reference data. The limitations of these LFT's were summarised in Table 1-2. Further evaluations of the methods and interpretative strategies are required to optimise the use of LFT's in the clinical management of lung diseases such as asthma and SCD.

1.13 Research Questions, Aims and Objectives

1.13.1 Primary aims

i. To establish appropriate methods for the application and interpretation of lung function measurements in children aged 4 to 12 years
ii. To determine the extent of ethnic differences in lung function between healthy Black and healthy White children after adjusting for height, sex and age.
iii. To determine the extent to which the various lung function outcomes (Impulse oscillation, Plethysmography and Spirometry) identify differences between healthy Black children and those with SCD

1.13.2 Secondary aims

i. To assess the extent to which data collection, quality control criteria and methods of reporting may contribute to within-test and between-test (or between-centre) variability of IOS, sR_{aw}, plethysmographic lung volumes and spirometry in children and, if necessary, develop revised recommendations and quality control criteria for these lung function tests.
ii. To investigate between-test repeatability of the various lung function outcomes and define thresholds for a significant bronchodilator response in school aged children.

1.14 Objectives

The primary objectives were to evaluate whether paediatric lung function reference data and guidelines for the selected tests were appropriate for use in healthy children aged 4 to 12 years by:

i. Recruitment of a large group of healthy children to ascertain the most appropriate published reference data for anthropometry, IOS, plethysmographic lung volumes and spirometry for use in children of Black and White ethnic origin
ii. Collation of sR_{aw} normative data from international centres to develop new reference equations.
iii. Assessment of the extent to which any bias occurred in results from healthy children when assessed using standardised protocols in different international centres or testing sites (e.g. schools vs. specialised laboratory conditions)

1.15 Hypotheses

i. All lung function outcomes are significantly different in healthy Black children when compared either with reference data derived from White children, or when directly compared to a contemporaneous group of healthy White children
ii. Currently recommended ethnic adjustment factors for spirometry and plethysmographic lung volumes are inappropriate for interpreting lung function in Black children.

1.16 Structure of the thesis

This thesis has nine chapters. Chapter 2 will describe the subjects, equipment and methods of the study. This will be followed by a brief results chapter defining the study population, four results chapters dedicated to each lung function test under investigation (IOS, sR_{aw}, plethysmographic lung volumes, and spirometry) and a final results chapter summarising the application of lung function tests in children with SCD. Chapter 9 forms the discussion of the thesis, the conclusions and directions for future work. All result chapters are self-contained, with detailed descriptions of the aims and objectives, hypotheses, a brief description of the specific study population, results and interpretation of results.

2. Subjects, equipment and methods

The previous chapter outlined the overall aims and objectives of the thesis. This chapter will summarise the subjects, equipment and methods for conducting this study. Based upon the literature review in chapter one, different areas for each test will need to be evaluated, hence specific research questions, hypotheses and sample size calculations will be included within the relevant chapters.

2.1. Inclusion criteria

- Healthy children (White and Black children) or
- Children with Sickle Cell Disease (SCD)
- Age range: 4 to 12 years
  - Lung volumes age range was limited to 6 to12 years
- All Children had fully informed consent from parents and assent from children to participate, which were specific to the research project through which they were enrolled (see Recruitment section: 2.3)

2.2. Exclusion criteria

- Failure to obtain consent
- Known concomitant diagnosis of lung disease (e.g. cystic fibrosis or asthma)
  - NB: children with SCD and asthma were included in the study
- Known heart disease that may result in surgical repair or catheter intervention after formal consultation with a cardiologist
- Born <37 weeks gestation / with history of neonatal lung disease / with low birth weight (<2500g)
- Any anatomical, spinal or thoracic abnormalities
- Known congenital abnormalities
- A recent respiratory tract infection within the last 3 weeks
- A recent acute or any significant chronic respiratory problems that have required hospitalisation or medication other than brief (<2 weeks) course of oral antibiotics
- Currently taking oral steroids

2.2.1. Medications to withhold and exclusion period

- Long Acting Bronchodilators for 12 hours
- Short Acting Bronchodilators for 8 hours
- Anti-histamines for 72 hours

2.3. Recruitment

Children were recruited through various research studies, the details of which are described in the follow sections.

2.3.1. SAC study

Children with Sickle Cell Disease (SCD) were recruited from a subset of the *Sleep and Asthma Cohort Study* (SAC study) which was established in 2005 (www.sacstudy.wustl.edu).

Available lists of neonatally screened and immigrating patients were used to identify eligible patients. Ninety-five per cent of patients in this age group regularly attended clinic, and those who did not were visited at home by the sickle nurse co-ordinator. All eligible children from three London hospitals (St Mary's, North Middlesex and St Thomas' were approached), thus sampling bias was minimised. The original inclusion criteria for this study included children aged 4 to 18 years, across three international centres (London (recruitment spanned three hospitals), St Louis, USA and Cleveland, USA. For the purpose of this study, only those children less than 12 years old with SCD were included.

The original SAC protocol did not have ethics to study healthy control children. In 2008, a substantial amendment was made to allow ethnically matched healthy control children to be studied to aid interpretation of the lung function results from children with SCD. Healthy children of Black origin who met the inclusion and exclusion criteria defined above were recruited and studied at the UCL, Institute of Child Health, London UK and at Washington University, St Louis, USA.

2.3.2. SLIC study

In 2010 the Asthma UK *Size and Lung function in Children* (SLIC) study commenced. This was a feasibility study which aimed to assess the impact of ethnicity and body composition on lung function in primary school children (5 to 11y). The overall aims of this study were to use state-of-the-art technologies to assess lung function and identify parameters of body size, composition, shape and physique that can better explain variability in lung function in health across all ethnic groups to facilitate interpretation of measurements in Black and ethnic minority children with lung disease such as Sickle Cell Disease. The longer term aims were to define simpler and cheaper methods of assessing body proportions and composition that could be routinely related to lung function, thereby overcoming the need for ethnic-specific reference ranges in clinical practice.

After gaining consent from the Local Education Authority (LEA) and the head-teachers, two inner-city London primary schools with a predominantly Black population were recruited. Science workshops were conducted for every class and all children received a recruitment pack (including patient information leaflets, questionnaires and consent forms). The study team returned on a later date to collect consent forms and conduct anthropometry and spirometry measurements on all children in whom consent had been obtained. At a later date, a subset of children were invited to attend the laboratory at the Institute of Child Health (ICH) to undergo more comprehensive lung function testing including measurements of impulse oscillometry (IOS), specific airways resistance (sR_{aw}), plethysmographic lung volumes and repeated spirometry.

2.3.3. Asthma UK

The Asthma UK Collaborative Initiative was established in 2005 to develop centile charts and investigate the impact of sex, age and body size on interpretation of lung function measurements (Spirometry, Interrupter Resistance Technique and Specific Airway Resistance) in young children by collating existing reference data and methodological details from centres around the world (www.growinglungs.org.uk). Reference equations have been successfully developed for Spirometry[126] and Respiratory Resistance from the interrupter technique.[127] The collaboration, investigation of the methods and the development of reference equations for plethysmographic sR_{aw} were included in this thesis and have also been published.[128] (NB reference equations were developed by Dr Sanja Stanojevic).

The collaborative group was initially comprised of members of the ATS/ERS paediatric pulmonary function test task force. Subsequently, collaborators were identified by systematically searching PubMed, advertising at international conferences, through membership bulletins, word of mouth and by hand searching relevant respiratory periodicals.

sR_{aw} data were collected in healthy children aged 3 to 11. All participating centres were asked to provide detailed information about recruitment, population characteristics, equipment and measurement protocols.

2.3.4. Other research projects

In addition to the prospective recruitment of healthy Black children to facilitate the interpretation of the lung function measurements in Black children, lung function data from White children from recent research projects at ICH were also evaluated.[16,104,129]

2.4. Ethics

Full ethical approval was obtained for each research project. All participants were given a recruitment leaflet appropriate for their age and information leaflets were given to the parents. Signed assent and parental consent was obtained from all participants. Examples of the patient information leaflets and assent/consent forms can be found in the appendix.

2.5. Equipment

All equipment used in this study was commercially available and met the minimum specification for lung function equipment as defined by the ATS/ERS taskforce.[39,94,105] In brief the following equipment utilised were:

1) Impulse Oscillometry: IOS Jaeger Masterscreen V4.65 (Wurzburg, Germany)
2) sR_{aw}: Jaeger Masterscreen body box (V.5.02)
3) Lung volumes: Jaeger Masterscreen body box V.5.02 (UK) or the Sensormedics V07-2B Box V6200 (USA).
4) Spirometry: Jaeger Masterscope V4.65.

2.6. Protocol

Health and safety assessments were undertaken prior to commencing each project, and study personnel underwent complete Criminal Records Bureau checks as well as attending Good Clinical Practice courses. All lung function measurements were conducted in a uniform way and were either undertaken in a primary school (spirometry assessments only) or within the laboratory (all lung function assessments).

2.6.1. School assessments

Recruitment packs were given to all children in two London primary schools during a science workshop. On the assessment day, those children with parental consent were taken out of class in groups of four. Children were assented and asked to complete a respiratory health questionnaire (appendix) with the help of an investigator. Anthropometry and spirometry measurements were conducted.

2.6.2. Lab assessments

On arrival to the laboratory, the procedures were explained to the parents and to the children. Information sheets had previously been sent to parents and children (see appendix). Consent and assent was obtained either at the laboratory, in clinic or (if part of the school visit) prior to coming to the laboratory. Anthropometry and measurements of IOS, sR_{aw}, plethysmographic lung volumes and spirometry were conducted.

2.6.2.1 Flow chart of laboratory lung function investigations

- Baseline IOS measurements (minimum of three, maximum of eight sets)
- Baseline sR_{aw} measurements (3 sets of 10 loops)
- 15 minute rest
- Repeat IOS measurements (minimum of three, maximum of eight sets)
- Repeat sR_{aw} measurements (3 sets of 10 loops)
- Baseline spirometry measurements (minimum of three attempts, no maximum amount of attempts)
- Plethysmographic Lung volumes (in children >6years; 3-6 attempts)
- Administration of 4 puffs of a bronchodilator (BD) (400µg Salbutamol) via a spacer (Aerochamber)
- 15 minute rest
- Post BD IOS (minimum of three, maximum of eight sets)
- Post BD sR_{aw} (3 sets of 10 loops)
- Post BD spirometry (minimum of three attempts, no maximum amount of attempts)

The duration of measurements was approximately 15 minutes for baseline measurements (IOS and sR_{aw}), 30 minutes for repeatability IOS and sR_{aw} measures plus baseline spirometry and lung volumes, and 25 minutes for post-bronchodilator response (IOS, sR_{aw}, spirometry). Due to time constraints and the length of the protocol it was not possible/feasible to conduct the entire protocol in all children, and parents were given the option to consent to selected parts of the protocol (i.e. baseline measurements only, baseline + repeatability/bronchodilator response, or complete protocol).

2.6.3 Anthropometry

2.6.3.1 Weight

Weight was measured in light clothing and without shoes to the nearest 0.1 kg using calibrated scales (i.e. a variety of weights were placed upon the scales to ensure accurate readings were made).

All measurements were undertaken on a level floor away from objects which may interfere with the measurements.

2.6.3.2 Height

Height was measured to the nearest 0.1 cm without shoes using a calibrated stadiometer (Harpenden Stadiometer, Holtain Ltd., Dyfed, UK in the laboratories, or the portable Leicester stadiometer (Crawlea Medical, Birmingham, UK) in the school). A 60cm measuring rod was used to ensure the validity of the stadiometer prior to every assessment day. Furthermore repeated height measures of study personnel were made using both stadiometers (wall-mounted (Harpenden) and portable (Leicester)) to ensure both stadiometers measured accurately.

The procedure was:

- Remove shoes and any hair ornaments which may interfere with the measurement
- Stand with feet flat on floor and heels against heel plate
- Back, shoulders, head, buttocks and calves against back board of stadiometer
- Head horizontal in Frankfurt (orbito-meatal) plane (see Figure 2-1)
- Operator placed hands under the child's ears to assist with posture
- Child breathed in then relaxed but remained in the tall position
- Height was recorded to the nearest 0.1 cm
- Measurement was repeated at least twice
- Repeated measures were within 0.1 cm

2.6.3.3 Sitting height

Where possible, measures of sitting height were obtained. Sitting height measures were similar to standing height measures:

- The subject was seated on a stool of known height
- Spine and bottom against the back board of the stadiometer
- Head was horizontal in Frankfurt (orbito-meatal) plane (see Figure 2-1)
- Hands on knees
- Child breathed in then relaxed but remained in the tall position
- Height was recorded to the nearest 0.1 cm
- Measurement was repeated at least twice
- Repeated measures were within 0.1 cm

2.6.3.4 Anthropometric reference data

Height, weight and body mass index (BMI: weight (kg) / height $(m)^2$) were converted to Z Scores based on both British White reference data,[130] and American *Centre for Disease Control* data based on White and Black children.[131]

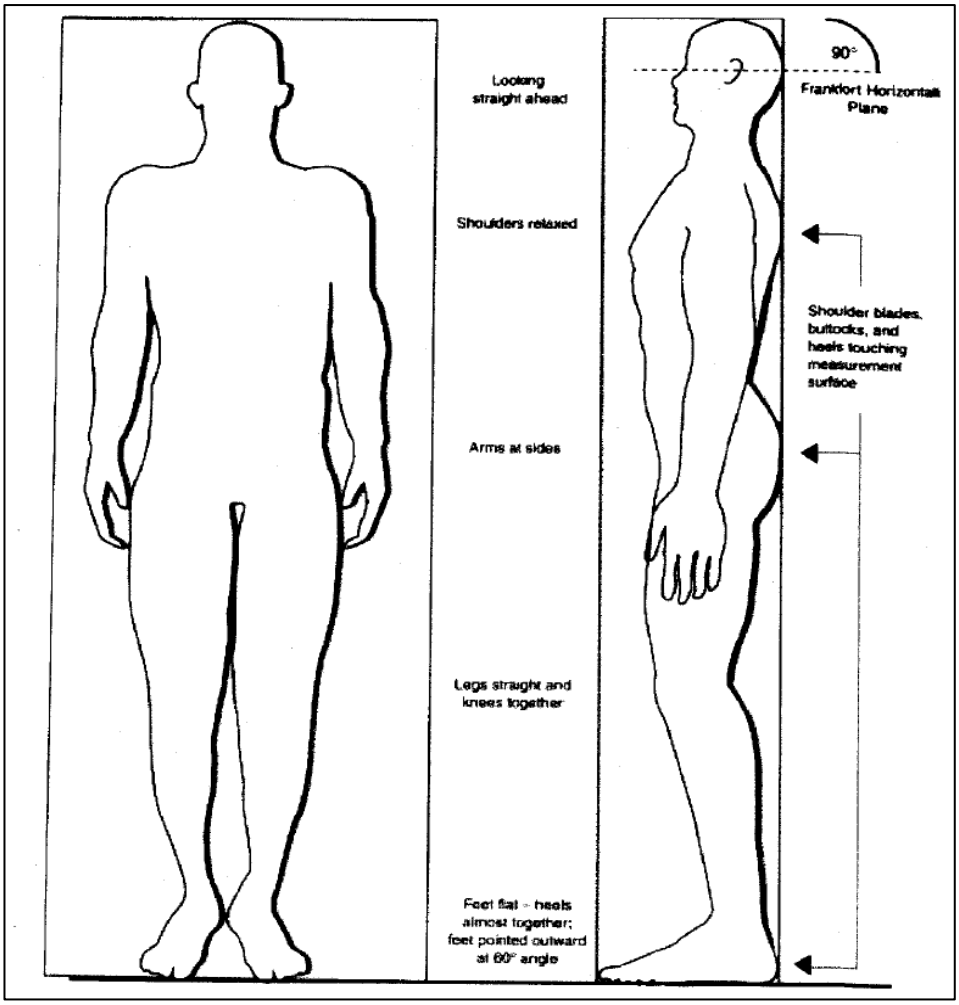

Figure 2-1: Standing position adopted during height measurements.

2.6.4 Impulse Oscillometry (IOS)

2.6.4.1 Equipment set-up and calibration

Prior to each assessment day, ambient conditions (temperature, barometric pressure and relative humidity) were recorded along with a volume calibration (a 3L syringe at multiple flows to ensure linearity of the pneumotach over a range of flows) and a pressure calibration with a reference impedance of 0.2 kPa·s^{-1} was conducted. A schematic diagram of the IOS set-up can be seen in Figure 2-2.

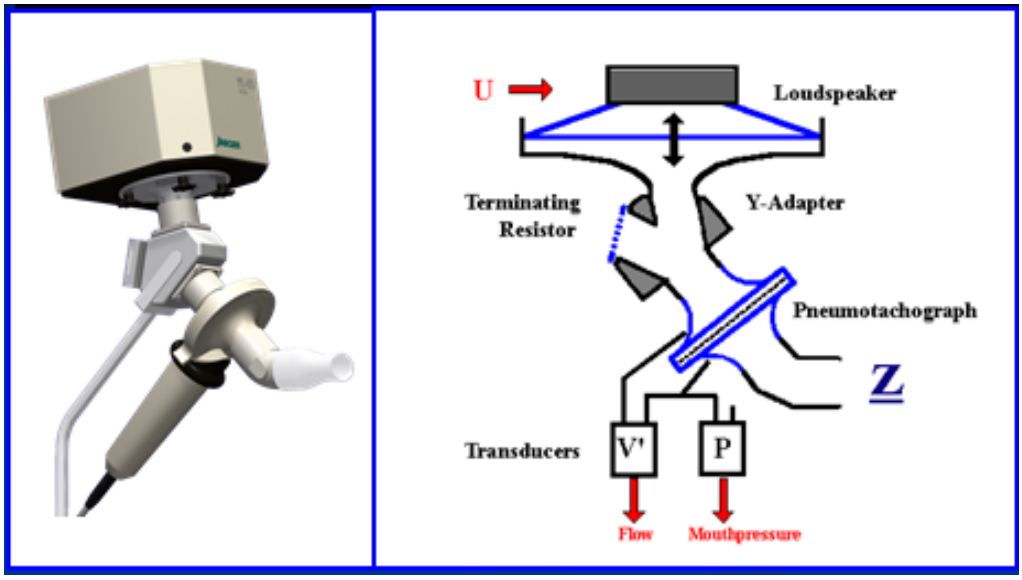

Figure 2-2: IOS equipment set-up

The child was connected, via a mouthpiece which incorporated a bacterial filter, to a set-up which utilised an external oscillating pressure from a loudspeaker. A pneumotachograph (PNT) measured flow (calculated from the pressure drop across a fixed resistance of screens), and pressure was recorded by pressure transducers. Both the flow created by the child as well as the pressure of the reflected waves coming from the lungs were recorded and the resistance (R_{rs}) and reactance (X_{rs}) of the respiratory system was calculated.

2.6.4.2 Outcome measures

A large array of IOS outcome measures was recorded in an attempt to identify the most appropriate outcome measure. The following outcomes were recorded:

- Resistance measured at 5Hz (R_5), 10Hz (R_{10}),15Hz (R_{15}) and 20Hz (R_{20}) ($kPa \cdot L^{-1}.s$)
- Frequency dependence of resistance between 5 and 20Hz (Fdr_{5-20}) ($kPa \cdot L^{-1}.s$)
- Reactance measured at 5Hz: X_5 ($kPa \cdot L^{-1}.s$)
- Resonant Frequency: F_{res} (Hz)
- Integrated area under the reactance curve: AX ($kPa \cdot L^{-1}$)

2.6.4.2.1 Impedance

The concept of impedance Z with respect to the lungs is a total opposition to breathing/flow. **Impedance of the Respiratory System (Z_{rs}) = Δ pressure / Δ flow** Z_{rs} is often considered a generalization of resistance, as it calculated by the pressure/flow relationship and includes the relationships of both the in-phase and out-of-phase pressure (P) and airflow (V'). However impedance is more complex than resistance because it represents the net sum forces that must be overcome to generate flow which include the resistive, Inertance and the visco-elastic forces that oppose respiration. i.e. Z_{rs} = Sum of the opposing forces (Resistance and Reactance) and in this thesis was expressed as either Resistance (R) or Reactance (X).

2.6.4.2.2 Resistance

Resistance of the Respiratory System (R_{rs}) is the component of the trans-pulmonary pressure in-phase with flow, or "real part" or the respiratory resistance. It represents the sum of viscous resistances of which airway resistance is the most significant. Low frequencies (5-10 Hz) penetrate the small airways (defined as bronchioles <2 mm in diameter), whereas high frequencies (15-20 Hz) remain in the upper airways. Therefore R_5 and R_{10} are thought to reflect resistance in the periphery and the central airways, and R_{15} and R_{20} are more indicative of resistance in the central airways. If there is significant peripheral airway oedema or bronchospasm, one would expect resistance in the peripheral airways to increase relative to the central airway (i.e. $R_5 > R_{20}$). Frequency dependence of resistance between 5 Hz and 20 Hz (Fdr_{5-20}) quantifies the change in resistance from the small airways to the central airways.

2.6.4.2.3 Reactance

Reactance of the Respiratory System (X_{rs}) is a complex quantity related to those portions of pressure oscillations out-of-phase with airflow, also termed the "imaginary part resistance." X_{rs} is determined by the elastic and inertive properties of the lung and undergoes a transition from negative values at low frequencies (elastic reactance

dominates) and increases to positive numbers at high frequencies (inertial reactance dominates).

Elastance (E) is the force required to overcome the elastic properties to expand the lung during inspiration. Hooke's Law states that the extension of a spring is in direct proportion with the load applied to it, in physiological terms, the elastic recoil of the lungs mean they spring back to their smallest size. After flow has occurred elastic forces are dominant and elastic forces increase with volume: Pressure / Volume (inverse of compliance). Hence, as the volume increases E becomes the dominant factor, and at smaller volume changes elastic forces are less important.

Inertance (I) is the force required to overcome the inertia (tendency of an object to resist a change in motion) and accelerate the gas (volume of air) into and out of the lung. Inertance describes the relationship between pressure and volume acceleration.

Elastance and Inertance may be separately visualized for clinical purposes however the frequency at which Reactance is zero is termed **Resonant Frequency (F_{res}).** F_{res} is a rough dividing line, whereby the low frequencies comprising those below F_{res} relate most prominently to elastic properties of peripheral airways, and high frequencies comprising those above F_{res} relate most prominently to the inertial properties of larger central airways.

Integrated Area of Reactance (AX) is a useful index to quantify changes in low frequency reactance. It is an integrated response index for reactance developed by Goldman reflecting the integral of the negative values of X from 5Hz to F_{res}.[38] This value may in part reflect small airway function.

2.6.4.3 Measurement procedure

The child was seated in an upright position with the head in neutral position, noseclips in place with the technician supporting the cheeks with their hands (to minimise upper airway compliance) (Figure 2-3).

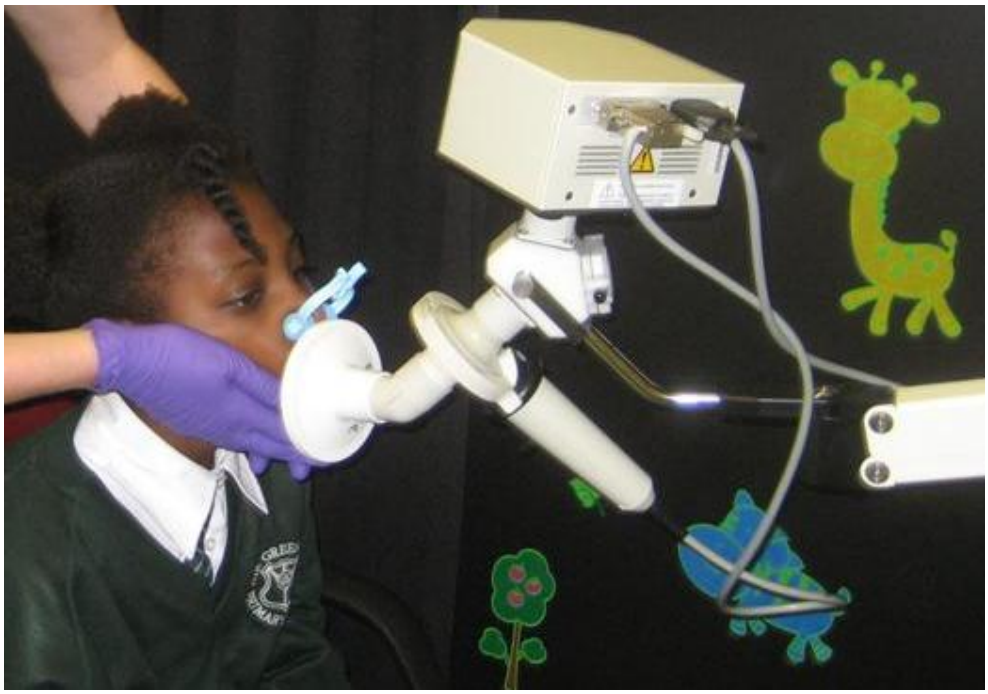

Figure 2-3: Child undergoing Impulse Oscillometry measurements.

The child was instructed to perform tidal breathing for at least 30 seconds (maximum 90 seconds). During this time the technician encouraged and reassured the child.

Online quality control included:

- Ensuring there was no movement in the mouth (chewing, talking etc.)
- Ensuring the tidal volume trace was stable, free from drift without hyper- or hypo-ventilation
- Observing the Z_5 trace to ensure consistency throughout the measurement (no obvious spikes in impedance that indicated swallowing, glottic closure or cough)

After completing the measurement the data were played back to undergo the quality control measures for assessing acceptability.

2.6.4.4 Quality control

An IOS QC criteria was developed to determine technical acceptability and data were given an over-read score according to (Table 2-1).

The volume-time tracing was reviewed to ensure tidal breathing remained stable throughout data acquisition. An example of unstable tidal breathing can be seen in Figure 2-4. Sudden drifts, reduction, plateaus or interruption on the volume tracing were suggestive of incomplete expiration, airflow leaks (either around the mouthpiece or due to improper seal with the noseclip), mouthpiece obstruction, coughing, glottic closure, breath-holding, swallowing, or vocalization and data were rejected/edited if disturbed by these artefacts. Furthermore "notching" on the volume tracing was an indication of airflow leak, and was detected by amplifying the volume trace. Although notching was acceptable at/near end inspiration and expiration (due to slow/no changes in volume), it was unacceptable if present on the upslope of inspiratory volume or the downslope of expiratory volume, and data were rejected/edited if airflow leak was evident.

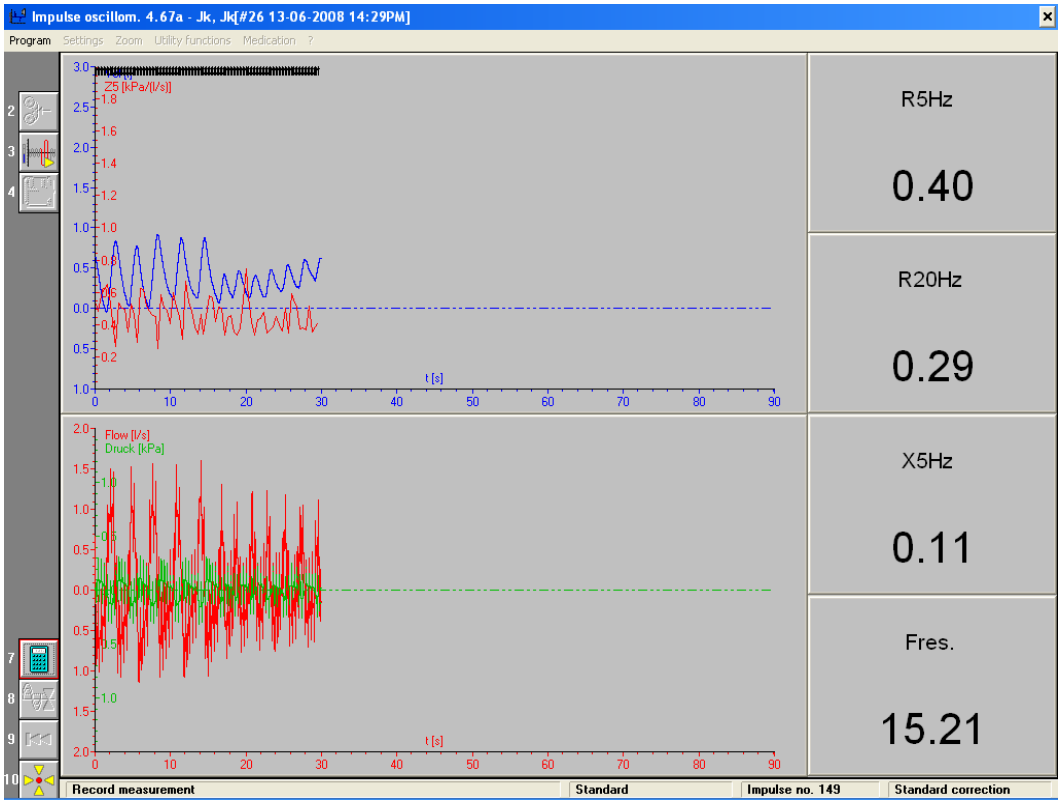

Figure 2-4: Screenshot of unstable tidal breathing during IOS measurements.
Legend: The first five breaths have a large tidal volume (900mL), next 7 breaths have small tidal volume. If the normal tidal volume for this subject was known, the data could be edited to include the acceptable portion.

Optimal Breathing frequency and tidal volume was within 15-25 breaths/min and 400-700mL respectively, however results were not rejected if outside the limits (although a lower QC score was awarded). Similarly, optimal coherence function (a number between 0 and 1, which provided an index of causality between the input and output of the linear system, and was therefore decreased in the presence of nonlinearities or extraneous noise) was >0.7 for coherence at 5Hz and 0.9 for coherence at 10Hz. The shape of the resistance and reactance slopes was also reviewed during QC analysis. It is physiologically implausible for R_5 to be less than R_{10}, hence if a "bump" in the resistance slope was observed the data were rejected. Similarly the reactance curve should move from negative values at X_5 to positive values after F_{res}. If X_5 was greater than X_{10} the slope was erroneous and was rejected. Finally the optimal data acquisition time was >20s with >4 breaths, however data were still included if >12s of data, with >4 breaths were recorded. Figure 2-5 illustrates unacceptable IOS measurements.

Figure 2-5: Screen shot of Unacceptable IOS data.
Legend: Poor quality measurement can be detected by the spikes in Z_5 (Red trace in the top box) indicating movement in the mouth, irregular tidal breathing (blue trace top box) and large changes in flow (Red trace bottom box).

Table 2-1: IOS over-read scoring sheet.

		1	2	3
Tidal breathing	Free from drift, stable and regular **(2)**			
	Free from drift, some irregular breaths **(1)**			
	Irregular breaths, evidence of drift **(0)**			
Notching	Free from “notching” and leak **(2)**			
	Mild notching present at end expiration **(1)**			
	Frequent notching **(0)**			
Breathing Frequency (bpm)	15-25 **(2)**			
	10-15 or 25-35 **(1)**			
	<10 or >35 **(0)**			
Tidal Breathing (ml)	400-700 **(2)**			
	250-400 or 700-850 **(1)**			
	<250 or >850 **(0)**			
Coherence at 10Hz	≥0.9 **(2)**			
	0.8 **(1)**			
	≤0.7 **(0)**			
Coherence at 5Hz	≥0.7 **(2)**			
	0.5 - 0.6 **(1)**			
	≤0.4 **(0)**			
R and X slopes expected shape	Both curves “expected” shape **(2)**			
	One curve “abnormal” shape **(1)**			
	Both curves “abnormal” shape **(0)**			
Acquisition time	≥20 sec and 4 breaths **(2)**			
	12 – 20 sec or 4 breaths **(1)**			
	<12 sec **(0)**			

2.6.5 Specific Airways Resistance (sR_{aw})

2.6.5.1 Equipment set-up and calibration

The Jaeger Masterscreen body box (V.5.02) was used for the assessment of sR_{aw}. Prior to every assessment day, ambient conditions (temperature, barometric pressure and relative humidity) were recorded along with a volume calibration with a 3L syringe at multiple flows (to ensure linearity of the pneumotach over a range of flows) (Figure 2-6).

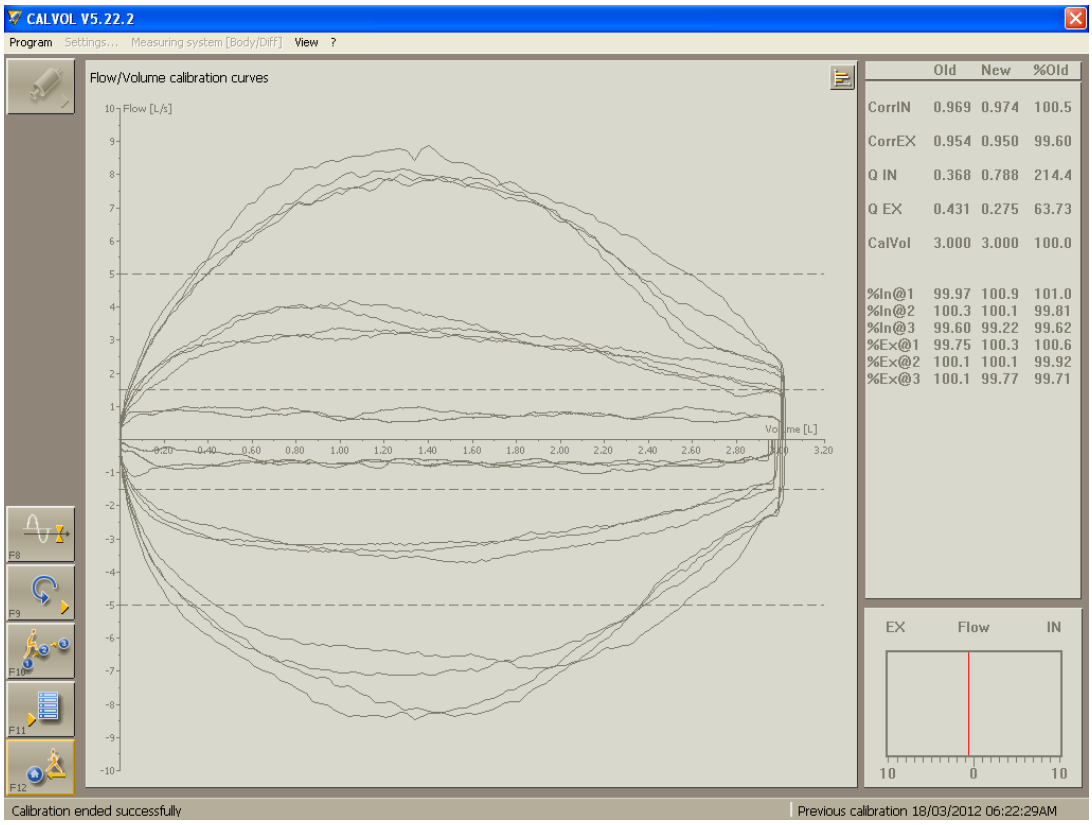

Figure 2-6: Volume calibration at multiple flows.

Box calibration consisted of a 2-minute stabilisation period with the door closed followed by a leak test in which the screen displayed the decaying signal which should be between 4 and 7 seconds (NB: if the half-life was less than 4.5 seconds, investigations such as checking if the door was closed properly, problem with the seal were conducted). The Jaeger software automatically repeated the leak test three times. If the range of half-lives from the three trials was greater than 1.5 seconds the calibration was repeated.

Following testing of half-life, the programme automatically provided a 50mL sinusoidal signal three times. The screen displayed the time-base plot, a bar chart of the 3 calibration factors (bottom right) and numerical data (top right). Consistency of the

calibration was ensured (i.e. all three columns were around 1±0.25) and stability of the signal on the time-base plot was examined by reviewing the 'QPB%' (the coefficient of variation) and ensuring it was <2% (Figure 2-7):

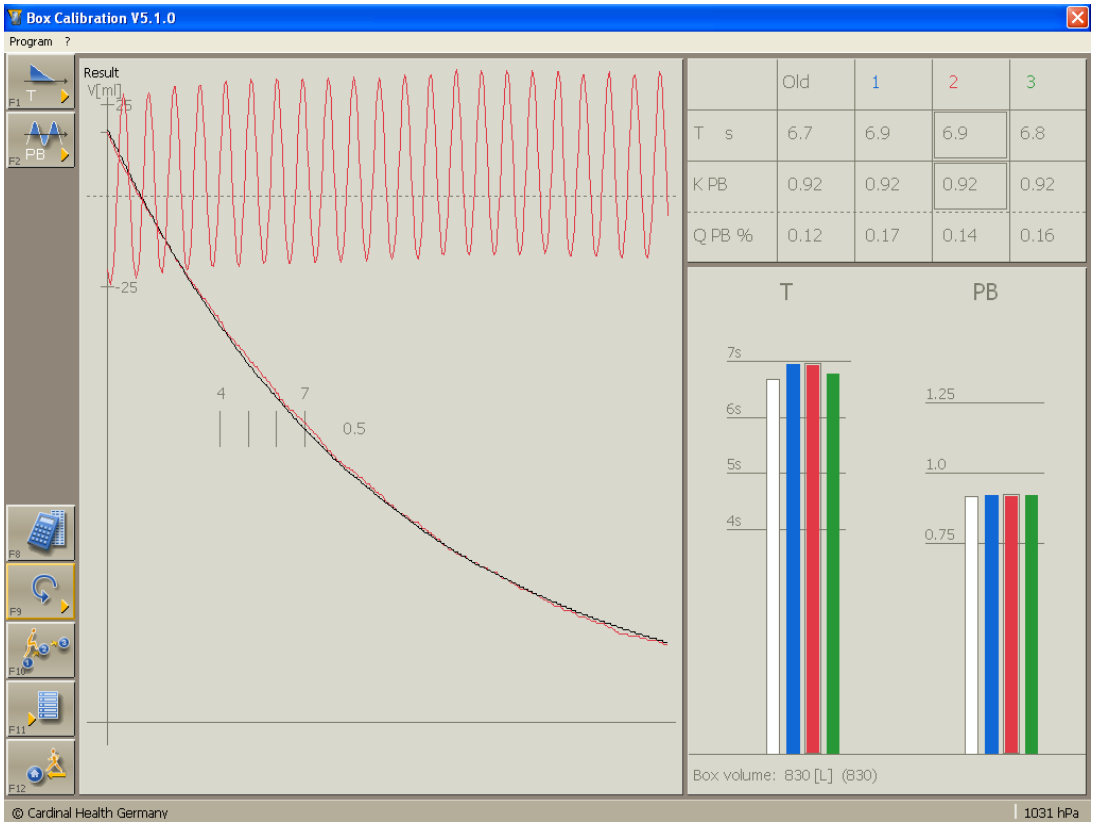

Figure 2-7: Box calibration.

2.6.5.2 Measurement procedure

The child was seated upright in the Jaeger body plethysmograph with their neck slightly extended to reach the mouthpiece (attached to a bacterial filter) and feet flat on the floor (or a step). The procedure was explained, and after one minute of rest with the door closed (to allow for thermal stabilisation) the child was instructed to breathe normally through the mouthpiece at a natural breathing frequency. Quiet breathing was encouraged and animations assisted maintaining a breathing frequency of 30 to 45 breaths/minute. Throughout testing, the child was wearing a nose-clip and supporting their cheeks with the palms of their hands (Figure 2-8). Three sets of 10 pressure-flow loops were recorded once a stable breathing pattern had been established.

Figure 2-8: Set-up of Body plethysmograph.
Legend: Light panel: child undergoing plethysmographic measurements with noseclip and hands on cheeks. Right panel: "Nessy" the computer animation that encourages natural breathing frequency.

2.6.5.3 sR_{aw} outcomes

The most suitable outcome measure was under investigation since derivation of sR_{aw} could be calculated from the relationship of plethysmographic (box) pressure to flow (P/F) in numerous ways: Figure 2-9 is a plot of a typical sR_{aw} loop, with change in respiratory flow on the Y-axis plotted against change in box (plethysmographic) volume (derived from box pressure) on the X-axis. P/F changes above zero flow represents inspiration, and below expiration. The dotted lines indicate inspiratory and expiratory flow at 0.5 $L.s^{-1}$. With the exception of 'effective resistance' (sR_{eff}), which was calculated as a regression of pressure and flow over the entire breathing cycle (Figure 2-9), the sR_{aw} outcome is generally derived from the tangent of the slope of P/F which can be placed:

- between peak inspiratory and peak expiratory flow (sR_{peak}). (Figure 2-10)
- between points of maximum plethysmographic (box) pressure (total resistance, or sR_{tot}) (Figure 2-11)
- over some fixed range of flow, over the central linear portion of the breath (most frequently between 0-0.5 $L.s^{-1}$ i.e. $sR_{0.5}$) (Figure 2-12)

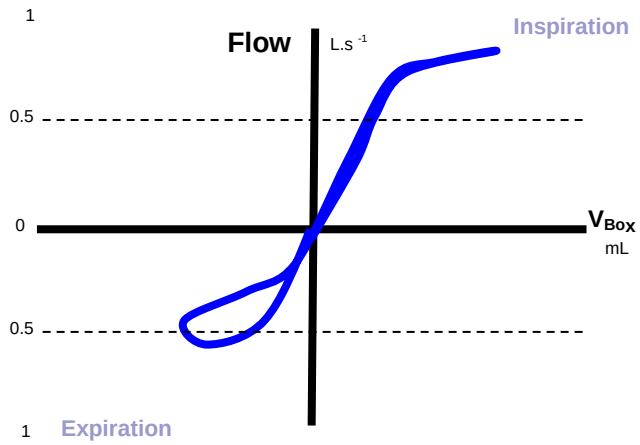

Figure 2-9: Effective sR_{aw} (sR_{eff})
Legend: Measured at each sample point of the breath.

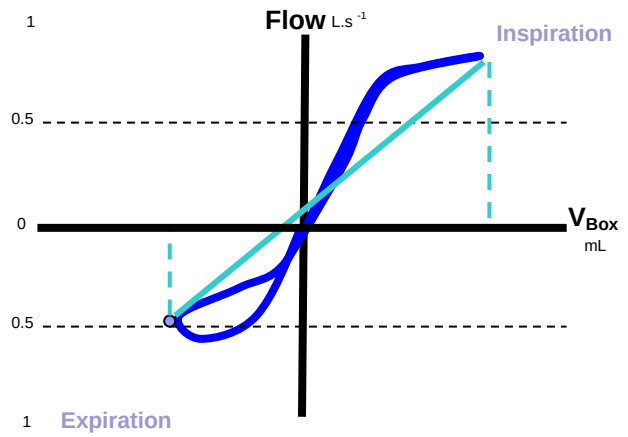

Figure 2-11: Total sR_{aw} (sR_{tot})
Legend: Measured between points of maximum pressure (i.e. box volume) swing during inspiration and expiration.

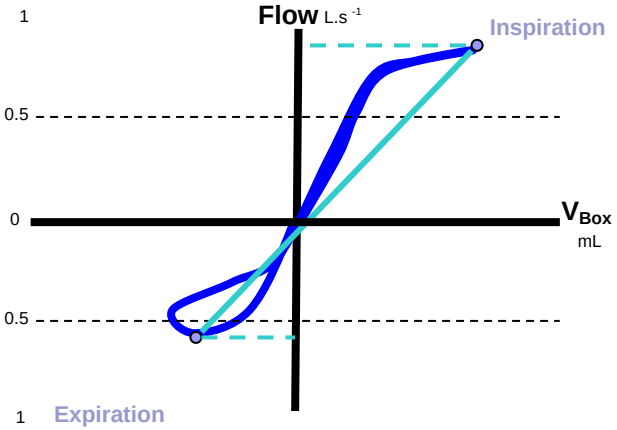

Figure 2-10: Peak sR_{aw} (sR_{peak})
Legend: Measured between points of peak flow during inspiration and expiration.

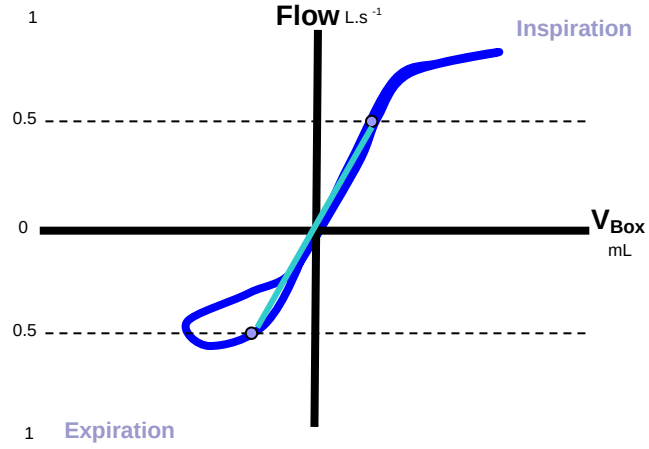

Figure 2-12: sR_{aw} at 0.5L.s^{-1} ($sR_{0.5}$)
Legend: Measured over the central, more linear, portion of the curve between inspiratory and expiratory flows of 0.5L.s^{-1}

2.6.5.4 Quality Control

Measurement protocols for data collected for the Asthma UK Initiative varied according to centre (these differences were investigated in Chapter 5)

In London, up to 5 trials of 10 or 5 breaths (dependent on software) were recorded with the aim of obtaining 3 'technically acceptable' trials, as defined by the following QC over-read criteria (Y = 1, N = 0):

- Respiratory rate between 30-45 bpm Y / N
- Breaths super-imposable (i.e. parallel slopes) Y / N
- Breaths of similar size and shape Y / N
- Breaths reasonably closed at zero flow Y / N
- No obvious distortions to the breath (e.g. glottic closure, cough, talking) Y / N
- More than one acceptable trial available Y / N

Figure 2-13 illustrates an acceptable sR_{aw} dataset which scored 6/6 on the QC score. Figure 2-14 and Figure 2-15 are examples of sR_{aw} data which had undergone QC over-reading and did not meet all the acceptability criteria, whereas Figure 2-16 is an example of sR_{aw} data which failed all categories of the QC Score.

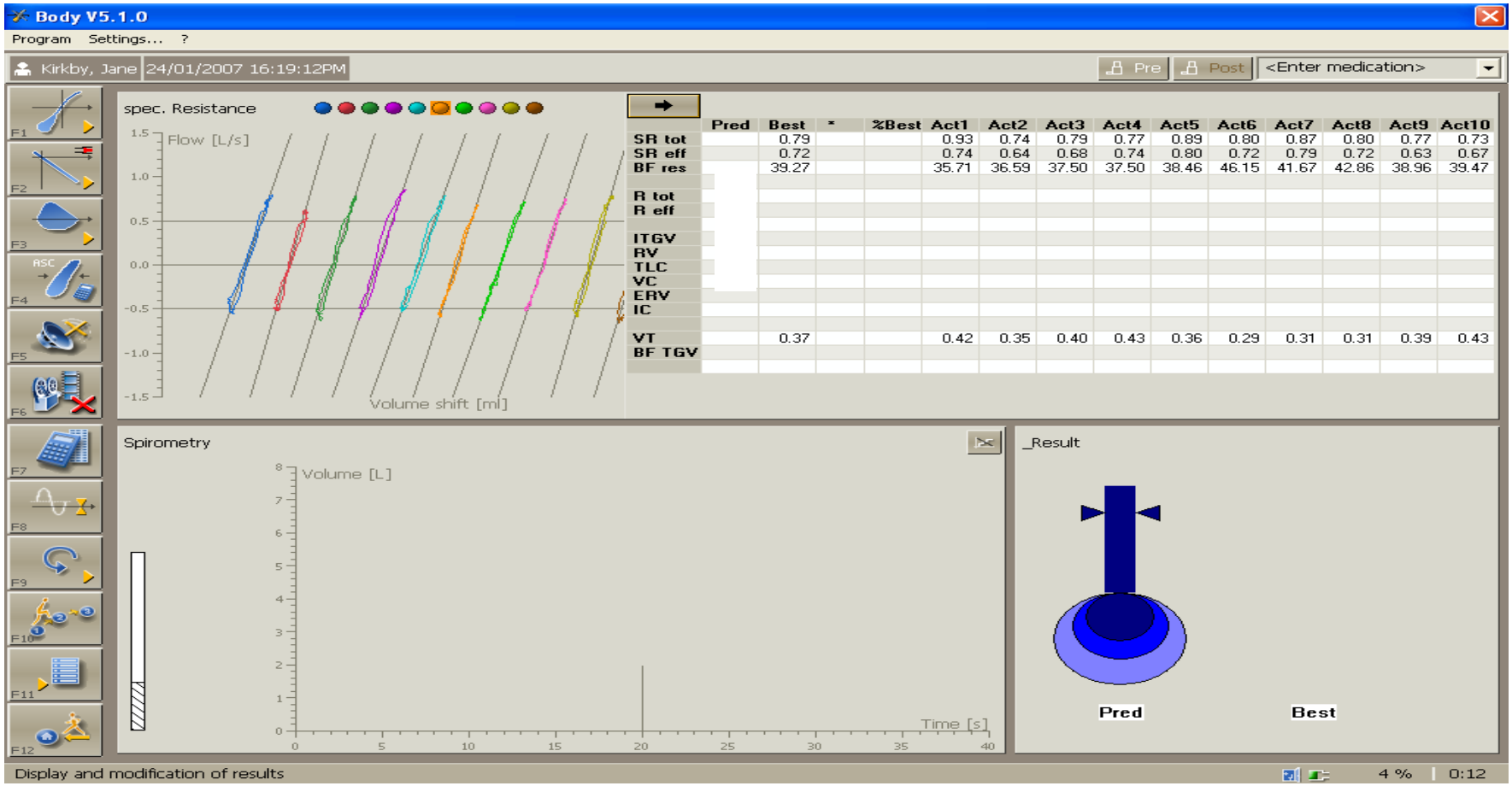

Figure 2-13: Example of an acceptable sRaw dataset with a QC score of 6/6
Legend: breathing frequency within 30-45bpm = Y; Superimposable = Y; similar size and shape = Y, closed at zero = Y, No distortion of breath = Y, came from set of 3 trials = Y. **QC score = 6/6.**

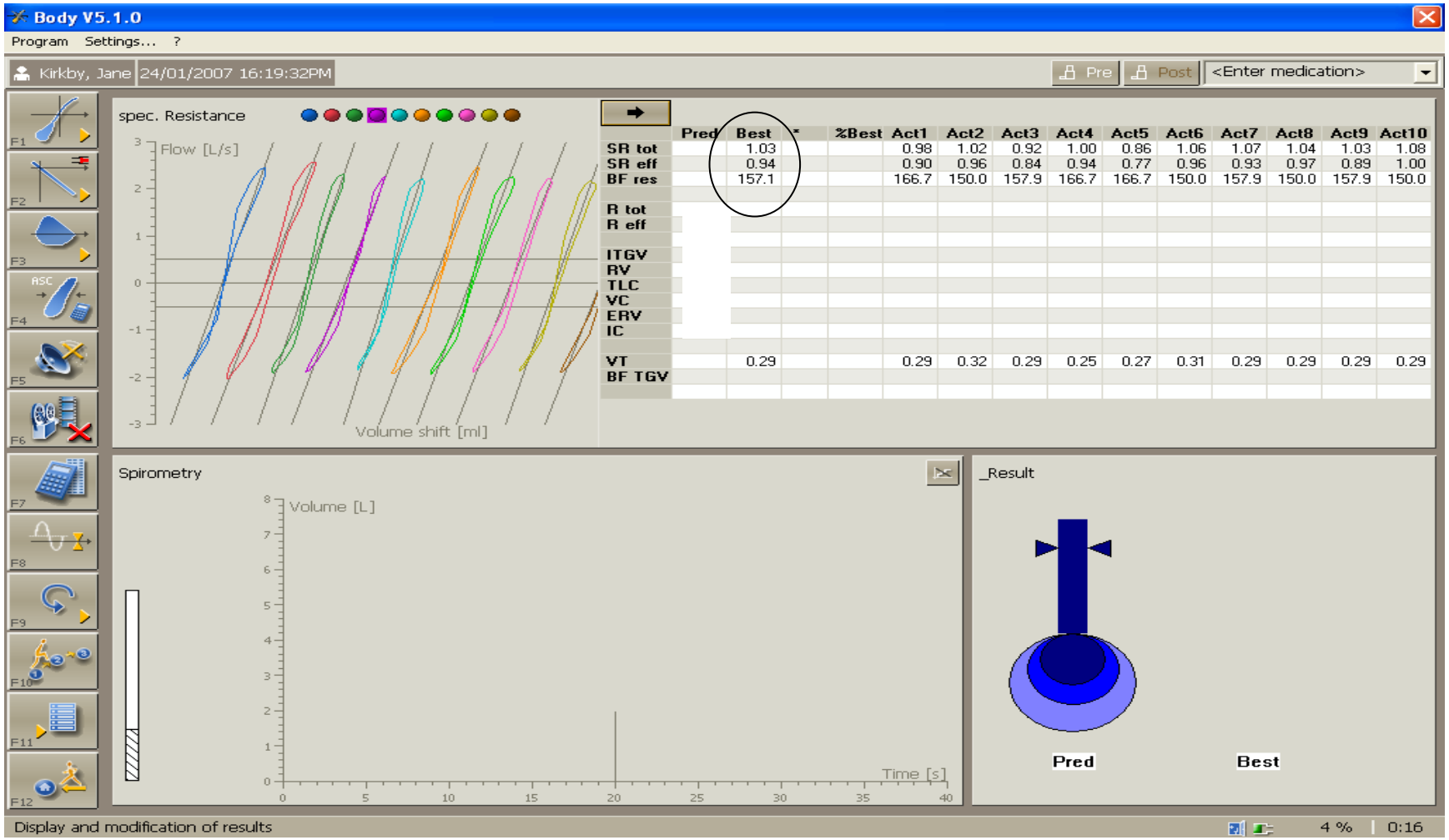

Figure 2-14: Example of a fast breathing frequency in sRaw measurements
Legend: Breathing frequency within 30-45bpm = N; Superimposable = Y; similar size and shape = Y, closed at zero = Y, No distortion of breath = Y; came from set of 3 trials = Y. **QC score 5/6**

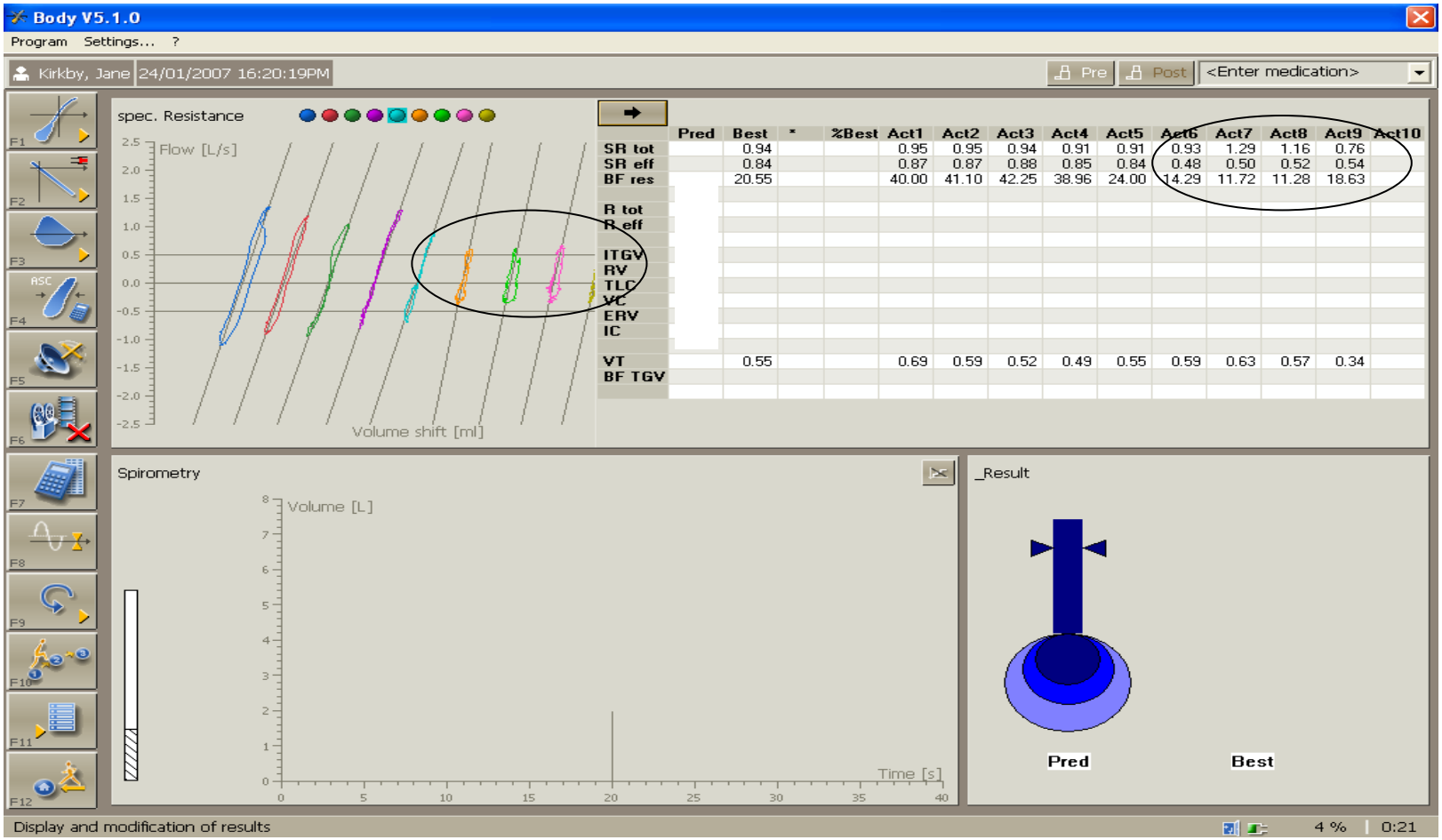

Figure 2-15: Example of sRaw data with a low QC score.

Legend: Breathing frequency within 30-45bpm =N; Superimposable = N; similar size and shape = N, closed at zero =N, No distortion of breath = Y, came from set of 3 trials = Y. **QC score = 2/6.**

Figure 2-16: Example of sRaw data which failed QC criteria
Legend: Breathing frequency within 30-45bpm =N (not reported); Super-imposable = N; similar size and shape = N, closed at zero = N, No distortion of breath = N, came from set of 3 trials = Y. **QC score = 1 /6. Unacceptable data which should not be reported**

2.6.6 Lung volumes

2.6.6.1 Equipment set-up and calibration

Measurements of plethysmographic lung volumes were undertaken immediately after those of sR_{aw} in the body plethysmograph, with measurement conditions the same as those described previously in section 2.6.4.1

2.6.6.2 Outcome measures

Figure 2-17 illustrates the partitioned lung volumes measured with spirometry and body plethysmography. The plethysmographic lung volume outcomes reviewed were:

- Functional Residual Capacity (FRC) (L)
- Residual Volume (RV) (L)
- Total Lung Capacity (TLC) (L)
- Ratio of RV to TLC (RV/TLC)

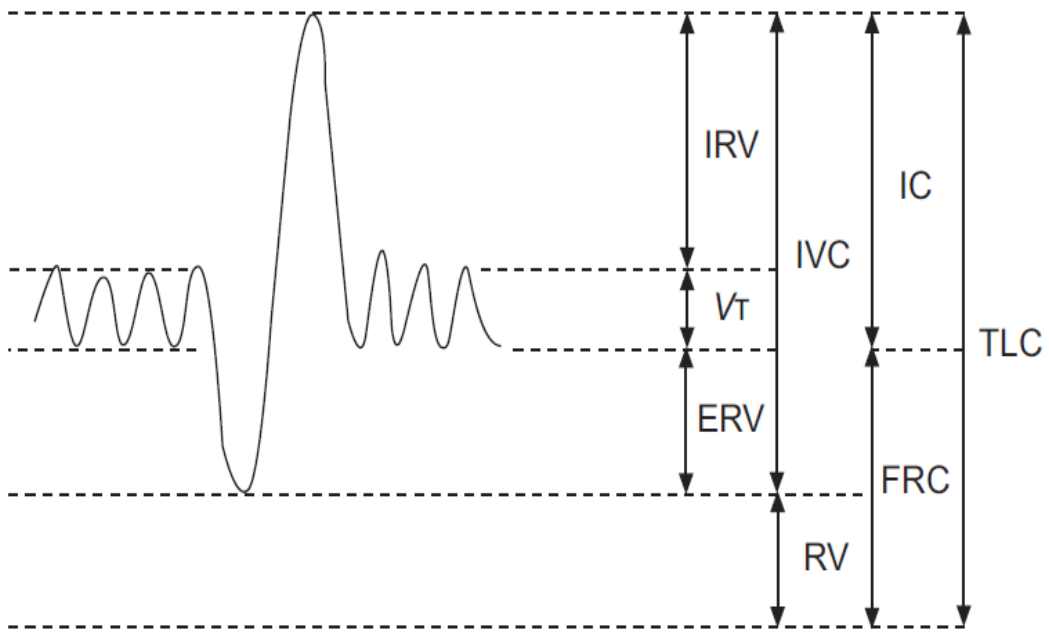

Figure 2-17: Spirogram identifying different lung volume levels.
Legend: IRV = Inspiratory Reserve Volume; V_T = Tidal Volume; ERV = Expiratory Reserve Volume;
IVC = Inspiratory Vital Capacity; RV = Residual Volume; IC = Inspiratory Capacity; FRC = Functional Residual Capacity; TLC = Total Lung Capacity

2.6.6.3 Measurement procedure

Plethysmographic lung volumes were performed according to a protocol based on the 2005 ATS/ERS recommendations.[94] Prior to commencing the measurement, simple instructions and time to practice were provided. The child sat within the body plethysmograph, after allowing for thermal stability (about one minute) the child went onto the mouthpiece with nose clips on his/her nose and their hands supporting their cheeks. After the consistent tidal breathing was established (i.e. sR_{aw} measurements were obtained), the shutter was activated at end expiratory level (FRC) and the child was instructed to continue breathing normally against the shutter for a series of 2-5 "technically satisfactory breaths" (see quality control, section 2.6.6.4). After the occlusion, the child took 1-2 tidal breaths and then a maximal breath to TLC, followed by maximal exhalation to RV, followed by another maximal breath back to TLC. FRC was calculated from the mean of 3-5 technically satisfactory FRC measurements, each of which consisted of at least two respiratory efforts at a breathing frequency of 30-90 breaths/min against the occlusion, with closed, superimposable loops free from artefact/drift). RV was calculated as the mean FRC minus the mean of the technically acceptable ERV measurements, and TLC was the reported value for RV plus the largest of the technically acceptable Inspired VC (Figure 2-17).

2.6.6.4 Quality Control

To ensure data collected met published guidelines,[94] and to document the quality of the results obtained, a plethysmographic lung volume over-read sheet was developed (Table 2-2). Plethysmographic lung volumes were graded according to three categories:

- Performance of FRC
- Repeatability of FRC
- Performance of spirometry

Each category was graded out of three, and a minimum of one for each category was required to "pass".

2.6.6.4.1 Performance of FRC

- Must have a stable end expiratory level, with no obvious drift in tidal breathing prior to occlusion
- At least 2 respiratory efforts against occlusion
- Breathing frequency during occlusion must be within 30-90 breaths/min
- Loops within each trial should be:
    - Free from artefact or drift
    - Closed and superimposable
    - FRC value for each individual loop should be similar (large differences will be inaccurate)

2.6.6.4.2 Repeatability of FRC

- Ideally >3 FRC within 5% or 100mL
- May accept
    - 2 FRC within 5%, or
    - 2 FRC within 10%, or
    - FRC within 10% with comment

2.6.6.4.3 Performance of spirometry

- The largest IVC ($\geq$85% of the previously recorded VC in spirometry) was recorded.
    - An acceptable FVC was required to calculate accurate TLC values. If an acceptable IVC was not recorded, values for TLC, VC and RV were not reported, however FRC could still be reported.
- Mean ERV was calculated from the same trials as the FRC
    - If the FRC was technically unacceptable the corresponding spirometry was excluded due to inaccuracies in the ERV values
    - An ERV measurement was excluded if it was clearly not representative and very different from the other measurements. In this case the mean of the remaining ERVs were used to calculate RV
    - A single ERV measurement could be used ONLY in the event that the VC trial it came from was technically acceptable and numerically similar to that previously recorded during spirometry

Table 2-2: Quality Control scoring system for plethysmographic lung volumes

	1) Performance of FRC $_{(pleth)}$ trial:	
1.1	≥ 3 technically acceptable trials *(i.e. ≥2 respiratory efforts against the occlusion, BF 30-90 breaths/min)*	**3**
1.2	2 technically acceptable trials	**2**
1.3	1 technically acceptable trial	**1**
1.4	< 1 technically acceptable trials and/or breathing frequency outside recommended range*	**FAIL**
	2) Repeatability of FRC $_{(pleth)}$ trials:	
2.1	≥3 FRC values within 5% or 100mls	**3**
2.2	3 FRC values within 10%	**2**
2.3	2 FRC values within 5%	**2**
2.4	2 FRC values within 10%	**1**
	3) Performance of Spirometry measurement: **IVC within 85% of previously recorded FVC AND…..**	
3.1	Mean of 3 technically acceptable ERV's	**3**
3.2	Mean of 2 technically acceptable ERV's	**2**
3.3	Single ERV measurement (compatible with previous FVC)	**1**
3.4	No technically acceptable VC measurement	**0**

*High breathing frequencies may be associated with hyperventilation and the subsequent elevation of FRC. Results with increased breathing frequencies therefore failed QC.

2.6.7 Spirometry

2.6.7.1 Equipment set-up and calibration

Spirometry measurements were obtained using identical spirometers and software in all sites (schools, UK laboratory and USA laboratory). The software included incentive spirometry to aid adherence and displayed real-time flow-volume and volume-time curves. (Figure 2-18)

Figure 2-18: Spirometry set-up and software incentives.
Legend: Child undergoing spirometry testing (left panel) and the incentive software to encourage maximal expiration (right panel)

In accordance with ATS/ERS recommendations,[105] prior to every assessment day, ambient conditions (temperature, barometric pressure and relative humidity) were recorded along with a volume calibration with a 3L syringe at multiple flows (to ensure linearity of the pneumotach over a range of flows).

2.6.7.2 Measurement procedure

Each spirometry session continued until three acceptable and two repeatable attempts had been achieved. The over-view protocol was published in *pediatric pulmonology*:[111] Quality control began with a visual inspection (rather than relying on customised software to "grade" the curves) to identify unacceptable curves. The following criteria based on the ATS/ERS 2005 guidelines,[105] modified slightly for children were then applied.

Start of test: Assessed by visual inspection of the flow-volume curve to ensure sharp peak flow, inspection of the volume-time curve to ensure no hesitancy, and a check to ensure back-extrapolated volume was <5% of the FVC and <150 mL.
Within test: Assessed to ensure the manoeuvre was free from artefact and free from cough within the first second.
End of test: Assessed by a visual inspection of the volume time curve to ensure an end-expiratory plateau had been achieved with no sharp cessation in expiratory flow. A minimum Forced Expired Time (FET) was not specified at the inception of this study, other than that this should be at least one second in duration.

2.6.8 Quality Assurance

Prior to commencing the study, all physiologists undertaking anthropometric and lung function assessments underwent identical training and were required to demonstrate adherence to the study protocol by both a written and practical examination. Furthermore repeated measures of the same subject by different physiologists ensured the physiologists were adhering to the protocol and that assessments were accurate.

Spirometry assessments were undertaken in three sites (London school, London laboratory and USA laboratory) and plethysmography was undertaken in two sites (London laboratory and USA laboratory). Each site had two biological controls who underwent monthly assessments to ensure equipment errors did not occur. Unfortunately, financial constraints did not allow a biological control to visit each international centre to compare equipment, however the consistency of the biological controls and the similarity of results from healthy children studied at the different centres (see section 6.6 and section 7.6.1) suggests no equipment bias.

2.6.9 Bronchodilator response

After completion of the baseline and/or repeatability tests, a short acting bronchodilator (salbutamol) was administered. The total dose was 400μg (4x100μg actuations) from a metered dose inhaler (MDI) through a spacer device (Volumatic). Young children were seated wearing a nose clip and breathing quietly. The operator held the spacer and instructed the child to breathe on the mouthpiece with tidal breaths sufficient to cause the valve to ‘click’. During a normal expiration the MDI was activated and the child continued to take a further ten tidal breaths. In older children the child was instructed to blow out until they were “empty” (i.e. RV) then take a maximal breath in and hold their breath for ten seconds, during the maximal breath the operator activated the MDI. (Figure 2-19)

Figure 2-19: Picture of a child taking a bronchodilator via a spacer.

A 30 second rest was given before repeating the process (tidal breathing method for young children, or breath-hold for older children) a further three times. Fifteen minutes after the bronchodilator administration "post BD" measures commenced.

2.7 Comparison to reference data

Published reference data for each LFT were applied to results obtained from healthy children to determine the appropriateness of the reference data in the healthy population.

IOS reference equations under evaluation were:

- Dencker *et al* (Scandinavian White children aged 2 to 11 years)[41]
- Nowowiejska *et al* (Polish White children aged 3 to 19 years)[65]

sR_{aw} data were collated to develop new reference data as part of the Asthma UK Initiative (Chapter 5).

Lung volume equations under evaluation were:

- Rosenthal *et al* (British White children aged 4 to 19)[98]
- Zapletal *et al* (Czech White children aged 6 to 17)[97]

Spirometry equations under evaluation were

- Stanojevic *et al* (Asthma UK, international collaboration of White subjects aged 3 to 80)[126]
- Wang *et al* (separate White and Black children aged 6 to 17)[5]

2.8 Data integrity and storage

In line with the Data Protection Act, all participants were given a unique, non-identifying subject ID. Contact details on study questionnaires were entered into a password protected database and were stored separately from the rest of the lung function data. In addition, all results were entered to a password protected database and double checked for errors. Lung function data were backed up, and electronic copies of all results and questionnaires were stored on a password protected computer, whilst hard copies were stored in locked cabinets.

Original IOS data and IOS data that had undergone "quality control analysis" were stored as PDF reports for each individual subject. The PDFs were converted to Microsoft Excel with "able2extract" software. Data for all subjects were merged, and the Data were coded by visit number (1 to 5), test status (1=pre BD, 2=post BD, 3 = repeatability) and attempt (3 to 5 attempts). The data was then converted to SPSS for analysis.

Original sR_{aw} data that were collated from five international centres were supplied in Excel format, and protocols regarding QC were supplied. Original sR_{aw}, lung volume and spirometry data collected in ICH (and spirometry data collected in London schools) were stored on the departmental database, from which data could be extracted into Excel format.

Original lung volume and spirometry data collected in the USA laboratories (SAC study) were double entered onto a database and extracted into Excel. A direct export from Jaeger spirometry software to the database was possible, however the lung volume data was over-read and manually entered onto a spreadsheet which was then uploaded onto the database.

2.9 Statistical analysis

Statistical analyses were performed using SPSS V18 (Chicago, USA), and Graph-Pad Prism V5 (San Diego, CA, USA). Each LFT was evaluated in terms of quality control, repeatability and reference data as identified by the literature review in Chapter 1.

2.9.1 Overview of analysis

Specific over-read sheets for each LFT were developed (section 2.6) and are discussed in the relevant chapters. Simple descriptive techniques were used to describe the over-read scores and regression analyses were used to assess the relationship between over-read score and age or disease. One-way ANOVA and independent t-tests were applied to assess group differences in different centres, differences between Black and White children and differences between health and disease, whilst Paired t-tests and Bland and Altman plots were used to assess within-test and between-test repeatability, and bronchodilator response. Direct comparisons of reference equations were performed using paired t-tests and limits of agreement between reference equations were determined with Bland and Altman analysis.

Statistical significance (expressed as p-values and 95% confidence intervals) identifies how likely that apparent differences between groups (e.g. health vs disease) are real and not due to chance. In all cases statistical significance was set at 5%, thus if the p-value was less than 0.05 the differences observed were likely to be real, and the chance of making a type 1 error (i.e. wrongly stating there was a difference when there was not a difference) was therefore 5% (1 in 20 samples). Since there was a 5% chance these conclusions were incorrect, the 95% confidence interval and the observed value were examined in relation to clinically important values. In addition, the potentialclinical significance (i.e. whether or not the magnitude of the observed difference was likely to be clinically important regardless or the statistical significance) was reported in each results section. The precise value of clinical significance (for example 0.5 Z Scores or 1 Z scores) was determined according to the specific research question and selected outcomes and is reported in each section.

2.9.2 t-tests

One sample t-tests were used to compare the mean of a continuous variable from a single sample against the hypothesised population mean. For example the mean sR_{aw} Z Score for healthy Black children was hypothesised to be zero.

Two sample t-tests were used to compare the means of two normally distributed populations. These tests were paired and unpaired. Paired t-tests were performed when the populations were equal, such as the mean R_5 before and after a bronchodilator. Unpaired/independent t-tests were performed when the samples were non-overlapping, for example comparing the mean FEV_1 Z score in Black children compared to the mean FEV_1 Z score White children. The assumptions were that the data were normally distributed.

2.9.3 Bland and Altman analysis

Bland and Altman analysis assessed the agreement between two repeats of continuous numeric measures (e.g. repeated LFT's) and gave the range within which 95% of the differences are expected to lie. The 95% limits of agreement (LA) was calculated as the mean difference +/- 2SD of the differences.[132] Measurements that fall outside the 95% LA were assumed to represent a clinically significant change/difference. For example if an outcome demonstrated limits of agreement of within +/-10% then differences exceeding 10% would provide a conservative estimate of a clinically relevant difference, that is, a difference that is likely to be caused by disease process/intervention rather than normal variability.

Bland and Altman plots were calculated graphically by plotting the mean difference of the repeated measures against the mean of the two repeated measurements against the calculated 95% LA. A clinically significant BDR was described as a response over and above that seen in the between-test repeatability assessment, and was estimated from the 95% LA determined by Bland-Altman analysis i.e. any points which outside the 95% LA meet the threshold of a significant BDR.

2.9.4 One way ANOVA

One-way analysis of variance (one-way ANOVA) was used to compare the means of two or more samples. The null hypothesis was that there is no difference between the groups. The ANOVA produces an F statistic which is the ratio of the variance calculated among the means to the variance within the samples. If there are differences between the group means, the variance between the group means should be lower than the variance of the samples. A higher ratio therefore implies that the samples were drawn from different populations and there is a difference between the group means. The assumptions of this test are that data are normally distributed, and come from independent samples.

2.9.5 Regression

Regression analysis was used to define a relationship between a dependent variable (e.g. lung function outcome) and one or more independent variables (e.g. age, sex, height, ethnicity). More specifically, regression analysis assesses how the typical value of the dependent variable changes when any one of the independent variables is varied, while the other independent variables are held fixed (i.e. the change in FEV_1 with every cm increase in height in boys aged 8 years old). Regression analysis also estimates the average value of the dependent variable when the independent variables are held fixed. Having met the assumptions for parametric analysis (i.e. data were normally distributed) simple linear regression analysis was used to determine the relationship between lung function outcomes and age/height and, where appropriate, to assess the relationship between different lung function outcome measures and determine correction factors if applicable.

2.9.6 Development of reference equations

Reference equations for sR_{aw} were developed by Dr Sanja Stanojevic using the LMS method.[133] The LMS is an extension of regression analysis which includes three components: 1) the skewness (**L**ambda), which models the departure of the variables from normality using a Box-Cox transformation, 2) the median (**M**u), and 3) the coefficient of variation (**S**igma), which models the spread of values around the median and adjusts for any non-uniform dispersion, hence LMS. The coefficient of variation (CV) is defined as (100 x SD/median). The three quantities (LMS) are allowed to change with height and/or age, to reflect changes in the distribution as children grow. The LMS method was applied using the Generalized Additive Models of Location Scale and Shape (GAMLSS) package in the statistical program R (Version 2.6.1; R Foundation, http://www.r-project.org)[133] Separate models were developed for males and females. A detailed description of the GAMLSS technique as it pertains to spirometry can be found in Cole *et al*.(2009).[134]

2.9.7 Comparison of LFT's

Since there was no gold standard LFT in this study, ROC (Receiver Operating Characteristic) analysis to determine optimal sensitivity/specificity was inappropriate. LFT's were analysed to describe the correlation between tests.

2.10 Sample size and power calculations

The broad research questions and hypotheses were set out in Chapter 1. Sample size calculations to detect specified differences with chosen power and significance were performed for each research question. The specified differences to be detected with confidence i.e. the minimum difference in lung function deemed to be of physiological importance was estimated from the literature or derived from the Bland and Altman Limits of Agreement calculated from repeatability data from pilot studies.

The "clinically important difference" was dependent upon the question being answered: For example when estimating the clinically relevant change required to determine a significant bronchodilator response, knowledge of the within-subject, between test repeatability (standard deviation (SD)) was required, whereas when attempting to distinguish health from disease, the between-subject repeatability (SD) within each group needed to be taken into account.

- A mean difference of 0.5 Z Score (0.5 SD) in lung function between tests was considered to be clinically significant
- A mean difference of 1 Z score (1 SD) in lung function between healthy children and those with SCD was considered to be clinically significant

The within-subject and between-subject SD for each LFT was required to perform the sample size calculations (Table 2-3) however, individual calculations for each LFT were not required as the calculations were based in SDs instead of absolute values. The ICH Statistics and Research Methodology CD calculator, "estimating with a specified precision for measures of limits of agreement" was used: A sample size of 48 was required to get limits of agreement within 0.5 SD with 80% power at the 5% significance level.

Table 2-3: Within-test and between test repeatability for each lung function test

	Within-test repeatability SD	Between-test repeatability SD
IOS: R_5 (kPa·s^{-1})	0.1	0.4
sR_{aw}: sReff (kPa·s)	0.1	0.2
Spirometry: FEV_1 (L)	0.15	0.5
Lung Volume: FRC (L)	0.15	0.4

Footnote: Repeatability was based on previous publications for each lung function test: IOS;[38] sR_{aw};[78] plethysmographic lung volumes;[98] and spirometry[117]

All sample size calculations were based on healthy children and estimated from the literature or pilot studies. Non-parametric tests would be required if the within-subject SD was different in disease compared to health (i.e. if the spread around the mean is greater in disease compared to health). If non-parametric tests are used the sample size needs to be increased by 16% to accommodate this. Therefore, a sample size of **56** children per group was required for this study.

3. Overview of results: Study population

3.1. Introduction: Study overview

The overall aims of this thesis were to establish appropriate methods for the application and interpretation of lung function measurements in children aged four to twelve years. Data from several studies were evaluated and where possible, data from healthy White and Black children were compared to establish if ethnic differences occurred and the applicability of published reference data. Given the different age ranges of studies included in this thesis (see section 2.1), the sample size and demographics of children studied varied for each lung function test. This brief results chapter will provide an overview of the study population. Four results chapters dedicated to each lung function test under investigation will follow, prior to a short summary results chapter.

In total, 214 healthy Black children and 186 healthy White children underwent spirometry assessments and subgroups of differing numbers also underwent other lung function measurements. In a separate retrospective study, 1908 sR_{aw} measurements from healthy White children studied in five international centres were evaluated (Chapter 5). One hundred and eighty of these subjects were measured in London and had matched spirometry/lung volume measurements. In addition to the healthy children assessed, 85 children with Sickle Cell Disease (SCD) from two international centres underwent lung function assessments (26 children underwent repeated sR_{aw} measurements). Table 3-1 summarises the subjects evaluated for each lung function test, and further demographic details are provided in the relevant chapters.

Table 3-1: Overview of the children who underwent each lung function test.

Test	Age (years)	Black (n)	White (n)	SCD (n)	Project*
IOS	4-11	68	0	59	SAC; SLIC
sRaw	4-10	56	1908	99	Asthma UK; SAC; SLIC
Lung Volumes	6-12	68	115	85	SAC; SLIC
Spirometry	6-12	214	186	60	SAC; SLIC

*Further information regarding each project/collaboration can be found in Chapter 2, section 2.3

Anthropometric outcomes (height, weight, and BMI) varied widely across the ages and ethnic groups (Figure 3-1).

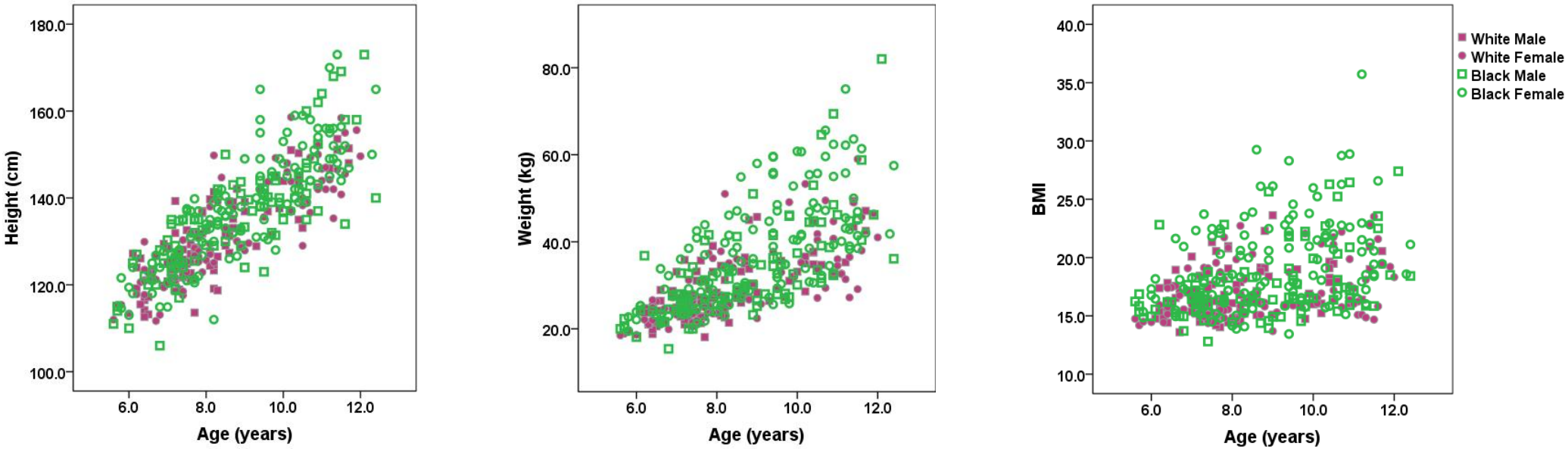

Figure 3-1: Anthropometry outcomes (height, weight and BMI) according to age.
Results based on 214 healthy Black children and 186 healthy White children.
Note the greater variability of height, weight and BMI at any given age among Black children than their White counterparts.

In order to compare growth patterns in healthy White and Black children, and subsequently compare healthy children to those with SCD, anthropometric data needed to be expressed as Z Scores to adjust for age and sex. Prior to performing any lung function comparisons, anthropometric data from children was expressed in relation to two sets of commonly used anthropometric reference equations to ascertain which were most appropriate to use when interpreting data from Black children with SCD.

3.2 Anthropometric reference data

In the UK, the British 1990 anthropometric reference equations[130] are commonly applied, whereas in the USA, the Centre for Disease Control and Prevention (CDC) 2000 reference equations[131] are generally used.

The British equations were developed from 17 distinct surveys of White children representative of England, Scotland and Wales.[130] Ethnic minorities were excluded *"on the grounds that they may grow differently from White children. The alternative, of producing ethnic-group-specific references, is even less satisfactory, as the required large and representative samples are simply not available, and the definition of ethnicity is in any case a minefield. A better answer would be to use a series of small-scale surveys to summarise the growth status of specific ethnic minority children in terms of their mean SDS on the British reference, and this could be used to recalibrate the reference for use with such groups."*

The CDC equations were developed from five national health examination surveys collected from 1963 to 1994 and five supplementary data sources.[131] These equations included ethnic minorities; the authors stated that: *"One issue that received attention is racial differences in growth. There are differences in size and growth among the major racial/ethnic groups in the United States, but these appear to be small and inconsistent. Therefore, the revised growth charts include all infants and children in the United States, whatever their race or ethnicity."*

The British anthropometry reference data[130] are therefore based entirely on White children, whereas the CDC reference data[131] were based on a mixture of ethnicities and potentially represent an "average" of Black and White children. A comparison of the two sets of equations was made prior to deciding which was the most appropriate for use in our population.

3.2.1 Comparison of anthropometric reference equations

Anthropometric data from 400 healthy children (214 Black children and 186 White) children were expressed as Z Scores using the British[130] or the CDC[131] equations. Regardless of the equation applied, the mean height, weight and BMI Z Scores from these children were significantly greater than the expected value of zero Z Scores (Table 3-2). Paired t-tests of values derived from the two equations revealed statistically significant differences between mean height, weight and BMI Z Scores, however these differences (~0.1 Z Scores) were not considered to be clinically relevant (Table 3-2).

Table 3-2: Comparison of anthropometric reference equations in 400 children.

	British 1990[130]	CDC 2000[131]	Mean Diff (95%CI) (British-CDC)	95% limits of agreement
White Children:				
Height Z Score	0.31 (0.99)	0.27 (0.93)	0.04 (0.02; 0.05) ***	-0.14; 0.21
Weight Z Score	0.39 (0.96)	0.32 (0.97)	0.07 (0.05; 0.09) ***	0.49; 0.96
BMI Z Score	0.32 (0.97)	0.28 (0.86)	0.04 (0.02; 0.07) ***	-0.26; 0.35
Black Children:				
Height Z Score	0.80 (1.23)	0.72 (1.14)	0.08 (0.06; 0.09) ***	0.56; 1.02
Weight Z Score	0.96 (1.15)	0.81 (1.03)	0.14 (0.13; 0.16) ***	-0.14; 0.43
BMI Z score	0.82 (1.19)	0.67 (1.00)	0.14 (0.11; 0.18) ***	-0.32; 0.61
Combined Black and White children:				
Height Z Score	0.57 (1.15)	0.51 (1.07)	0.06 (0.07; 0.05)***	-0.15 ; 0.27
Weight Z Score	0.70 (1.11)	0.59 (0.99)	0.11 (0.10; 0.12)***	-0.17; 0.40
BMI Z Score	0.59 (1.10)	0.49 (0.89)	0.10 (0.08; 0.12)***	-0.31; 0.51

Unless stated otherwise, results presented as mean (SD), ***p<0.001
Comparison based on 214 Black children and 186 White children aged 4 to 12 years.

Despite the clinically insignificant mean differences between the two equations, Bland and Altman analyses revealed relatively wide limits of agreement and a significant positive bias (Figure 3-2). Z Scores calculated by the British[130] equations were greater than those calculated with the CDC equations,[131] with the magnitude of the difference being greatest in those with the highest Z Scores for any outcome and in Black children who had significantly higher height, weight and BMI Z Scores compared to their White peers.

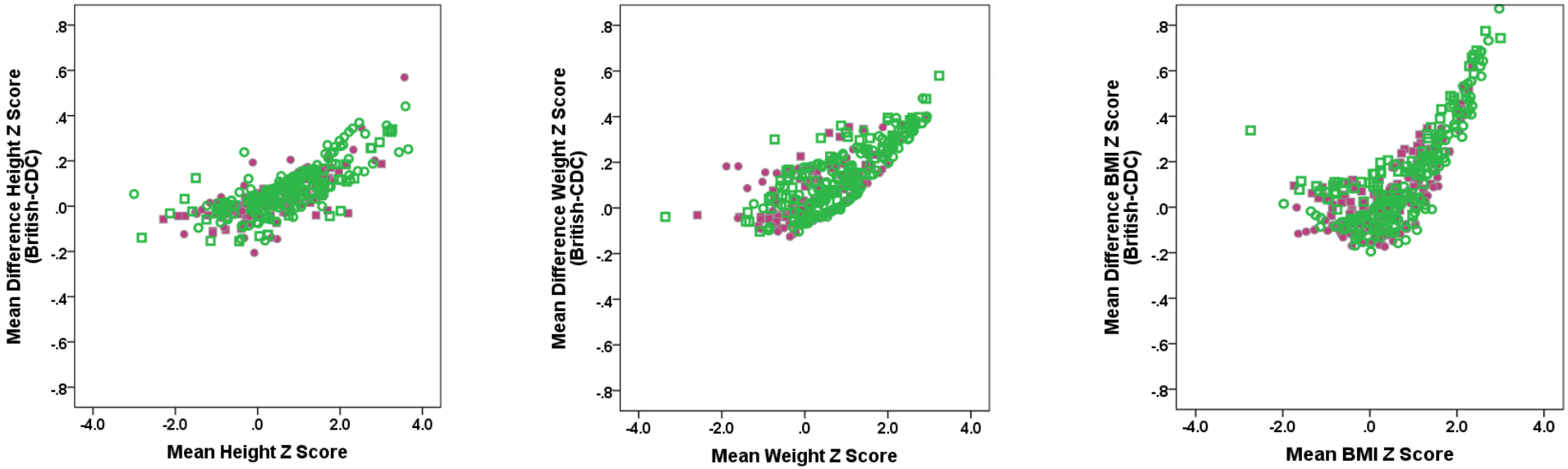

Figure 3-2: Bland and Altman comparison of height, weight and BMI Z Scores.
Z Scores calculated from the British 1990[130] and CDC 2000[131] equations.
Legend: Purple = White children; Green = Black children; Squares = males; circles = females.
A positive bias occurred in all outcomes. The bias was greatest in the children >2 Z Scores (who were generally Black). Z Scores calculated using the British[130] equations were generally higher than those calculated by the CDC equations.[131]

3.2.2 Anthropometric reference data: Impact of ethnicity

Figure 3-2 highlights the bias between the British[130] and the CDC[131] anthropometry reference equations, with the greatest differences occurring in those children with the highest Z Scores (who were predominantly Black children). For example, a 9 year old Black girl, 165 cm tall had a calculated height Z Score of 5.0 when applying the British[130] equations and 4.3 Z Scores using the CDC[131] equations, a difference of 0.7 Z Scores. A similar example using weight occurred in a 6 year old Black boy weighing 36.8 kg where weight Z Scores were calculated as 3.5 Z and 2.9 Z Scores for British[130] and CDC[131] equations, respectively. Finally, an 11 year old Black girl with a BMI of 35 kg/m^2 had a BMI Z Score of 3.6 Z by British[130] and 2.6 Z by CDC equations.[131] Independent t-tests revealed a significant ethnic difference in the mean differences between the two equations (Table 3-3), such that in White children either reference equation could be applied, whereas larger discrepancies between the two equations were observed in Black children.

Table 3-3: Differences observed between two anthropometric reference equations.

	Black: Mean diff (British-CDC)	**White: Mean Diff (British-CDC)**	**Mean Diff (95%CI) (Black – White)**
Height Z Score	0.08 (0.12)	0.03 (0.09)	0.05 (0.02; 0.06)***
Weight Z Score	0.14 (0.15)	0.07 (0.12)	0.07 (0.05; 0.10)***
BMI Z Score	0.15 (0.24)	0.04 (0.15)	0.11 (0.06; 0.14)***

Unless stated otherwise, results presented as mean (SD), ***p<0.001
Footnote: Anthropometric reference data based on British 1990[130] and CDC 2000[130,131] equations, applied to 214 Black children and 186 White children aged 6 to 12 years.

3.2.3 Summary of anthropometric reference data

Anthropometric outcomes (height, weight, and BMI) varied widely across the ages and ethnic groups. Such differences could have potential clinical implications as anthropometry, in particular height, has been shown to have an important influence on lung function outcomes.[8]

The British[130] anthropometry reference equations were based on White children only, whereas the CDC[131] equations included ethnic minorities. Bland and Altman analysis of 214 Black children and 186 White children' anthropometric details expressed as Z Scores using the two equations revealed a significant positive bias, with higher Z Scores being calculated with the British 1990 equations. The largest discrepancies between the two equations occurred in Black children, whereas White children had closer limits of agreement. In summary, the CDC 2000[131] anthropometric reference equations were more appropriate for use in this population which included both White and Black children. Therefore, the CDC 2000[131] equations have been used to calculate anthropometric data throughout this thesis.

4 Impulse Oscillometry

4.1 Introduction

Impulse oscillometry (IOS) is an effort independent measurement of total respiratory resistance shown to be a useful research tool for assessing airflow obstruction[45,67-71] and bronchodilator responsiveness.[37,72,73,135] The clinical usefulness of this technique is, however, limited due to a lack of reference data particularly in non-White children, insufficient guidelines,[1,39,124] and poorly defined repeatability and thresholds for bronchodilator responsiveness (BDR). This chapter will review the available IOS reference data and evaluate the use of these data in healthy Black children and children with Sickle Cell Disease (SCD). Quality control (QC), repeatability, BDR and other factors which may influence IOS measurements will also be investigated.

4.2 Aims

The primary aims were:

i. To establish appropriate methods for the application and interpretation of IOS in children aged 4 to 12 years
ii. To assess the extent to which IOS results from healthy Black children agree with published reference data (which is based on White children)[41,65]
iii. To determine the extent to which the various IOS outcomes identify differences between healthy Black children and those with SCD

The secondary aims were:

i. To assess the extent to which data collection, quality control criteria and methods of reporting may contribute to the within-test and between-test variability of IOS and to develop revised recommendations and quality control criteria for IOS measurements
ii. To investigate the between-test repeatability of the various lung function outcomes and define thresholds for a significant BDR in school aged children

4.3 Objectives

The primary objectives were to evaluate whether published paediatric reference data (based on White children) and guidelines for IOS were appropriate for use in Black children. This was done by recruiting a group of healthy Black children and a group of Black children with SCD and undertaking IOS measurements according to a standardised protocol, based on current methodological recommendations[1,39,124] (see section 2.6.4).

4.4 Hypothesis

IOS data from healthy Black children will be significantly different to that predicted by reference data derived from White children.

4.5 Subjects and sample size

Impulse oscillometry data were collected in healthy Black children participating in the SAC study and SLIC study (described previously in chapter 2, section 2.3). Children older than eleven years of age were excluded since the reference equations by Dencker *et al* were limited to children younger than eleven years.[41] Power calculations demonstrated that IOS measurements from 64 healthy children would enable differences equivalent to 0.5 SD to be detected between the controls and the published reference data (based on White children) with 90% power at the 0.05 significance level.

4.6 Reference data

IOS data were collected in 68 healthy Black children (41% male, mean (SD) age: 8.2 (1.5) years). The following outcomes were recorded:

- Resistance measured at 5Hz (R_5), 10Hz (R_{10}),15Hz (R_{15}) and 20Hz (R_{20}) ($kPa \cdot L^{-1}.s$)
- Frequency dependence of resistance between 5 and 20Hz (Fdr_{5-20}) ($kPa \cdot L^{-1}.s$)
- Reactance measured at 5Hz: X_5 ($kPa \cdot L^{-1}.s$)
- Resonant Frequency: F_{res} (Hz)
- Integrated area under the reactance curve: AX ($kPa \cdot L^{-1}$)

Low frequencies (5-10Hz) penetrate the small airways (defined as bronchioles <2mm in diameter), whereas high frequencies (15-20Hz) remain in the upper airways. Therefore R_5 and R_{10} are thought to reflect resistance in the periphery (and the central airways) and R_{15} and R_{20} are more indicative of resistance in the central airways. Fdr_{5-20} quantifies the change in resistance from the small airways to the central airways. Reactance is the out-of-phase resistance which reflects the energy storing

capacity of the airways. Low frequency reactance (X_5) is dominated by elastic forces within the airways, while the frequency at which reactance is zero (F_{res}) describes the point at which the elastic and inertial forces are equal in magnitude. AX is the integral of the negative reactance values (X_5 to F_{res}) and therefore quantifies changes in low frequency reactance (and hence may reflect small airway function). Increased AX (due to increases in X_5 and/or F_{res}) may reflect peripheral airway obstruction.

Absolute values against height can be seen in (Figure 4-1). A relationship between height and all IOS outcomes (with the exception of F_{res} which was relatively stable in this age range) was observed, therefore in order to interpret these results, a comparison to published reference data which adjusted for height was performed.

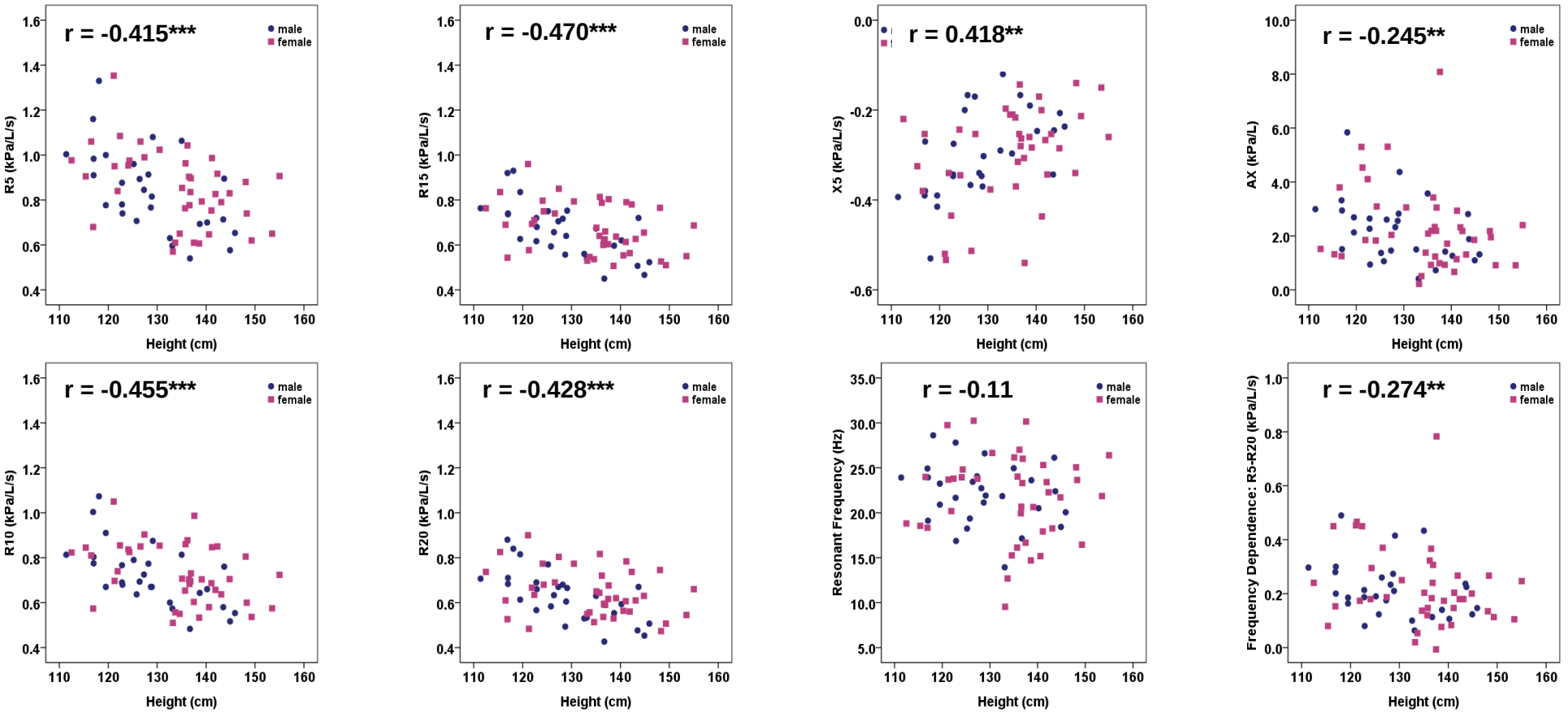

Figure 4-1: IOS outcomes plotted against height.
Results based on 68 healthy Black children aged 6 to 11 years.
With the exception of Resonant Frequency, all IOS outcomes were significantly, albeit weakly, correlated with height. No Sex differences were observed. **p<0.01, ***p<0.001

4.6.1 Published reference data for IOS

Two paediatric reference equations (based on White children) by Dencker *et al*[41] and Nowowiejska *et al*[65] were evaluated. The Dencker[41] equations were developed in 2006 from two collated populations (109 Finnish pre-school children aged 2 to 7 years, and 251 Swedish children aged 7 to 11 years). The calculated reference equations from all 360 children spanned an age range of 2 to 11 years (height range: 90-160cm), and used height and weight to calculate predicted values for resistances measured at 5, 10, 15 and 20Hz, reactance at 5Hz, and resonant frequency (F_{res}). RSD values were provided to enable the calculation of Z Scores, and limits of normality were defined as +/-1.96 Z Scores. Similarly, Nowowiejska *et al*[65] provided equations and RSD values derived from 626 Polish children aged 3 to 19 years to calculate predicted values and Z Scores for the same IOS outcomes however, these equations were based on height alone.

Neither set of equations adjusted for age, stating the use of height eliminated the need for age in the equation. In addition, both found that sex made no impact on the between-subject variation within each IOS outcome. Nowowiejska provided sex-specific (to match that of other lung function assessments) and sex-combined equations, whereas the Dencker equations only provided sex-combined equations. No sex differences were observed in the investigated population (Figure 4-1). For comparative purposes, the sex-combined equations were evaluated.

NB: At the time of writing this thesis, there were no reference data available for AX or Fdr, and no Black-specific IOS reference data were available.

4.6.2 Comparison of reference data

Two paediatric IOS reference equations[41,65] could be compared using results from 68 healthy Black children (41% male, mean (SD) age 8.2 (1.5) years). Differences for all IOS outcomes were apparent, regardless of the equation applied (Figure 4-2 and Figure 4-3). When applying equations by Dencker, measured values of resistance were on average ~0.5 Z Scores (15-20% predicted) higher than predicted. Similarly, mean F_{res} was ~2 Z Scores (~30% predicted) higher and mean X_5 was ~0.8 Z Scores (~40% predicted) lower than predicted. Significantly greater differences were observed when the Nowowiejska[65] equations were applied (Table 4-1).

NB: Although both Dencker and Nowowiejska developed reference equations for reactance measurements, only the equations by Dencker produced physiologically plausible results, (X_5 Z Scores being in the range of -30 to -8 Z Scores when calculated by Nowowiejska). Comparisons between reactance Z Scores was therefore not possible.

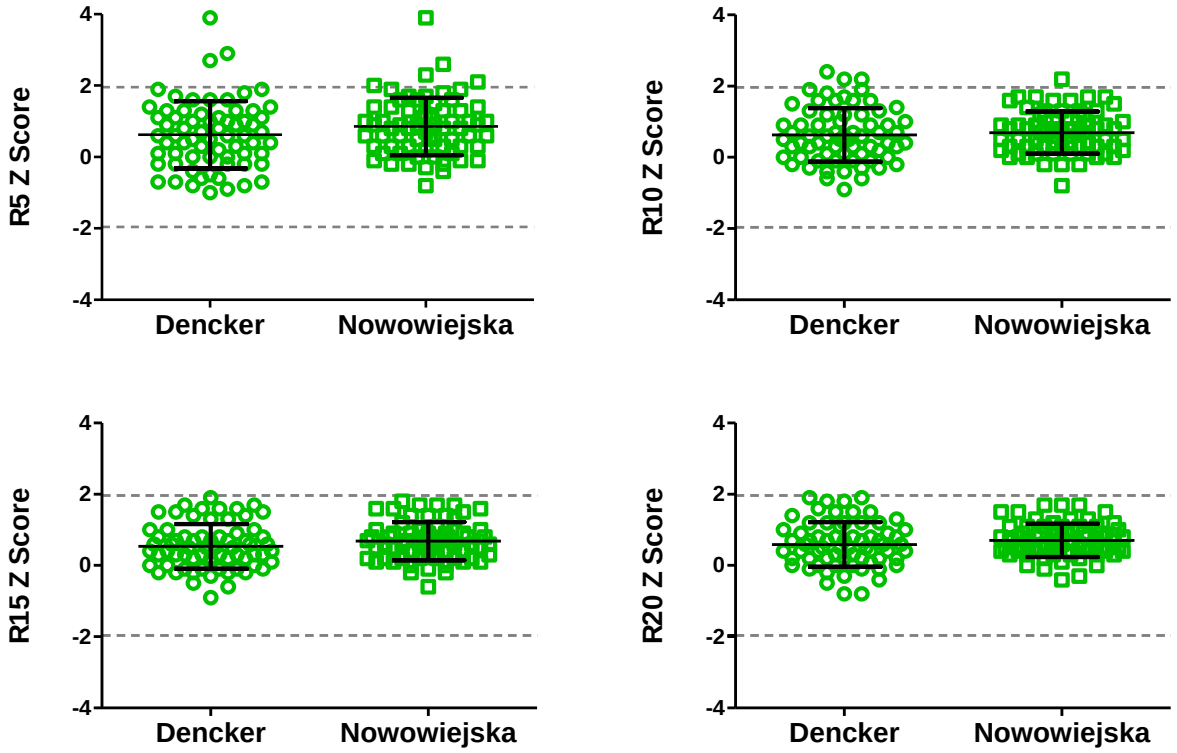

Figure 4-2: Comparison of IOS resistance data according to two reference equations
Legend: Results expressed as Z Scores using Dencker[41] and Nowowiejska[65] equations. Black lines denote mean +/-SD for the studied population. Dashed grey lines indicate the limits of normality (+/-1.96SD) for the reference data. Significant differences between Z Scores calculated from each equation were observed. In all outcomes the mean was significantly higher than the expected zero Z Scores, however the majority of results fell within the expected +/-1.96 SD.

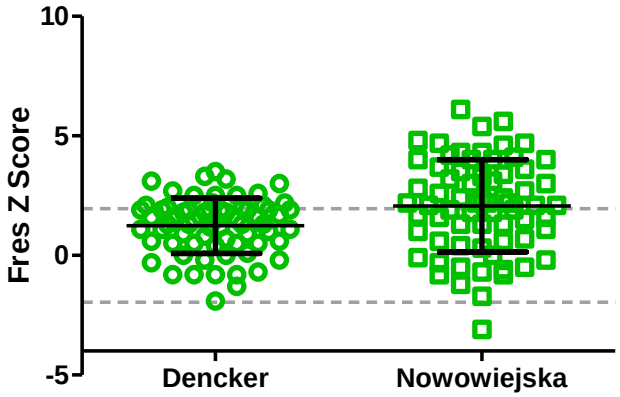

Figure 4-3: Comparison of F_{res} data according to two reference equations
Legend: Results expressed as Z Scores using Dencker[41] and Nowowiejska[65] equations**.** Black lines denote mean +/-SD for the studied population. Dashed grey lines indicate the limits of normality (+/-1.96 SD) for the reference data
Significant differences between the two equations were observed. In addition, a large SD around the mean highlighted the extremely high variability associated with this outcome measure which was potentially underestimated by Nowowiejska *et al*

Table 4-1: Comparison and limits of agreement of IOS outcomes.

	Mean (SD) Den	Mean (SD) Now	Mean Diff (SD) (Den. – Now.)	95% Limits of Agreement
R_5 Z Score	0.63 (0.94)	0.85 (0.81)	-0.22 (0.40)***	-1.00; 0.55
R_{10} Z Score	0.63 (0.75)	0.70 (0.59)	-0.07 (0.32)	-0.71; 0.58
R_{15} Z Score	0.54 (0.64)	0.68 (0.53)	-0.14 (0.15)***	-0.43; 0.14
R_{20} Z Score	0.59 (0.63)	0.70 (0.46)	-0.11 (0.17)***	-0.45; 0.23
X_5 Z Score	-0.81 (1.12)	-	-	-
F_{res} Z Score	1.23 (1.16)	2.01 (1.93)	-0.78 (1.10)***	-2.99; 1.33

Unless stated otherwise, results presented as mean (SD) ***p <0.001
Results expressed as Z Scores according to Dencker (Den);[41] and Nowowiejska (Now)[65]

4.6.3 Bland and Altman comparisons of IOS reference data

With the exception of R_{10} Z Score, statistically significant differences between the two equations were observed. With the exception of F_{res} Z Score, these differences were relatively small (0.1-0.2 Z Scores) with narrow limits of agreement (Table 4-1). The closest agreement occurred at higher frequencies where within-test variability (SD) was lowest. Despite the small mean difference and narrow 95% limits of agreement observed, Bland and Altman plots for these resistances revealed a slight bias at R_{15} and a greater bias at R_{20} suggesting the two sets of equations are not comparable (Figure 4-4).

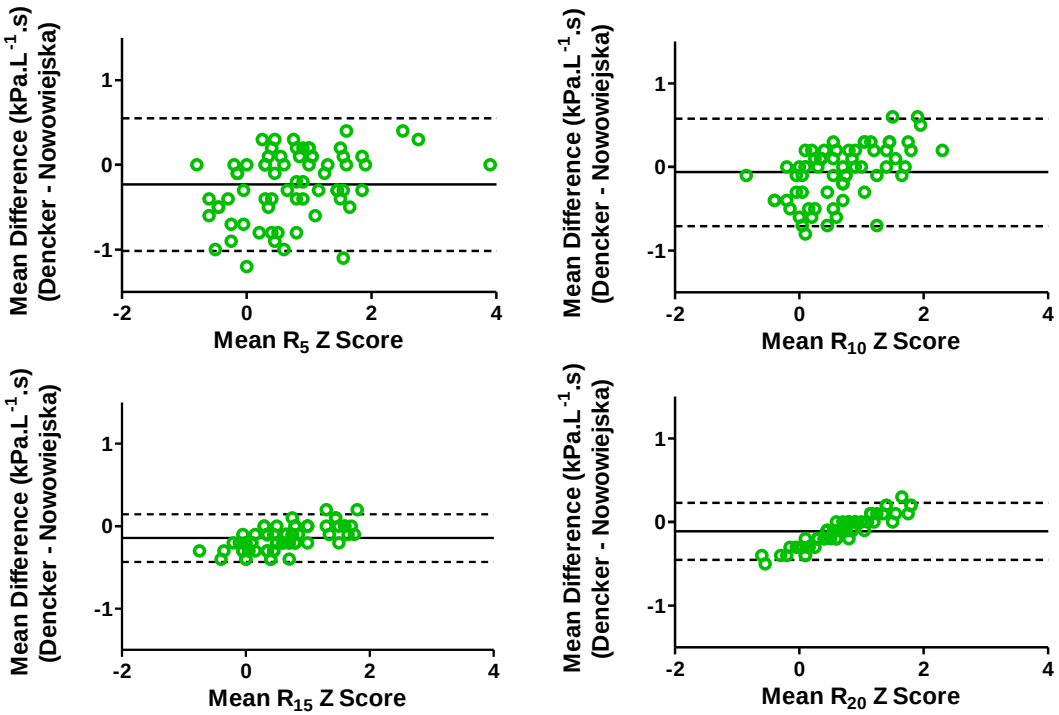

Figure 4-4: Bland & Altman plots for IOS resistance at different frequencies.
Legend: Results based on 68 healthy Black children aged 6 to 11 years and expressed as Z Scores using Dencker[41]and Nowowiejska[65] equations.
solid line denotes the mean difference. Dashed lines denote the 95% limits of agreement.
Relatively narrow limits of agreement were observed, however, a bias was apparent in all outcomes; hence the two equations were not comparable, and results would be interpreted differently according to the reference equation applied.

Larger mean differences and 95% limits of agreement where observed when comparing F_{res} Z Scores, and a large negative bias was apparent, such that in taller children differences of up to 4 Z Scores occurred depending on the reference equation applied (Figure 4-5).

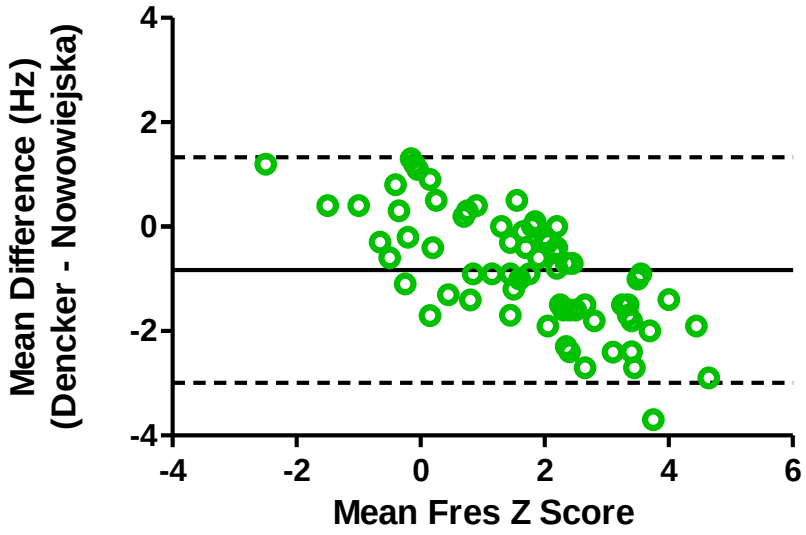

Figure 4-5: Bland and Altman comparison of F_{res}.
Legend: Results based on 68 healthy Black children aged 6 to 11 years and expressed as Z Scores using Dencker[41] and Nowowiejska[65] equations.
solid line denotes the mean difference. Dashed lines denote the 95% limits of agreement.
A large negative bias was observed such that a F_{res} result may be within the normal limits according to Dencker and below the normal limits according to Nowowiejska.

The bias observed in R_{15} and R_{20} Z Scores may be due to the different independent variables in each reference equation. Dencker[41] equations were based on height and weight, whereas Nowowiejska[65] equations were based on height alone. The larger bias seen in F_{res} Z Scores was a result of the contradicting predicted relationships between height (and weight) and F_{res} (Table 4-2). Dencker[41] predicted F_{res} to be relatively stable with increasing heights, with the F_{res} decreasing slightly when heights and weights were greatest; whereas Nowowiejska[65] predicted a negative association with height (Figure 4-6). Given such large differences in predicted values the two equations were not comparable.

Table 4-2: Reference equations by Dencker and Nowowiejska for F_{res}.

Dencker:	**Nowowiejska:**
$11.749+(4.379*(height,cm)^{-3})+(0.103*weight)$	Exp (-0.0101*height,cm + 4.164)

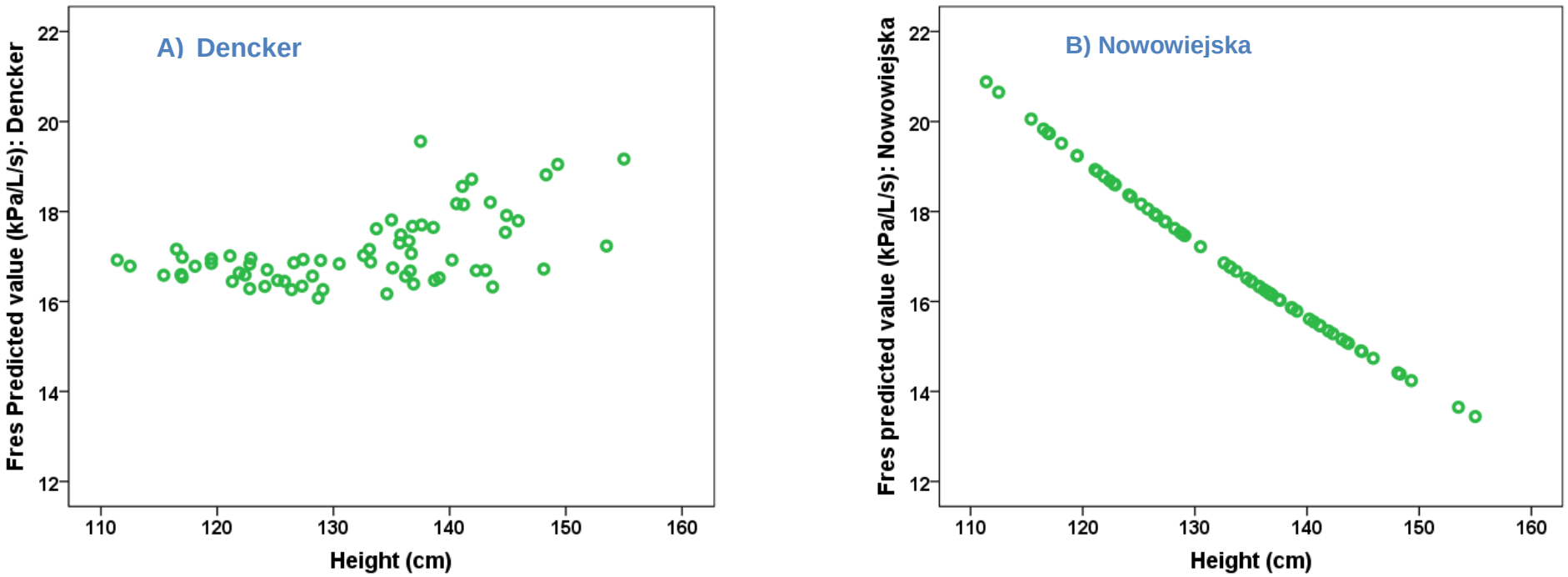

Figure 4-6: Predicted F_{res} values according to two paediatric equations.
Legend: Figure A represents predicted values based on Dencker[41] equation which was based on height *and* weight and hence demonstrate increased variability. Figure B applies the Nowowiejska[65] equation which was based on height alone.
Footnote: The two equations have contrasting predicted relationships between height (and weight with respect to Dencker) and F_{res}.

4.6.4 Summary of reference data

The potential applicability of two paediatric IOS reference equations derived from White children[41,65] was examined. Bland and Altman analysis revealed adequate, albeit with a slight bias, limits of agreement when comparing resistance results according to these two equations, but large discrepancies when comparing F_{res}. These discrepancies were due to the contrasting relationship between height and F_{res} described by each author (Nowowiejska described a negative relationship, and Dencker described a more linear/slight positive relationship). The two equations were therefore not interchangeable. The relationships between IOS outcomes and height observed in the studied population of healthy Black children most closely reflected that described by Dencker *et al*,[14] therefore these equations were selected as the most appropriate of the two reference data sets for use in our population.

Interpretation of IOS data in healthy Black children was evaluated using a direct application of the Dencker[14] reference equations and the predefined limits of normality of +/-1.96 Z Scores. In a normal, healthy population the mean (SD) is expected to be 0 Z Scores (1). The mean resistance measured at all frequencies were ~0.5 Z Scores higher than expected, however the narrower SD (0.6-0.9) around the mean meant that the majority (96%) of healthy Black children assessed fell within the predicted range (+/-1.96 Z Scores) for all resistance measurements. The lower SD in the studied population suggests that the between-subject variability in the original reference population was very large.

When reviewing X_5 results obtained in healthy Black children, a mean (SD) reduction of 0.8 (1.1) Z Scores was observed, and 8 children (12%) had X_5 results below the lower limit of normal. Z Scores calculated by Dencker[14] for resistance and reactance may be adequate for healthy Black children so long as caution is applied when defining limits of normality. Investigation of F_{res} in healthy Black children using the Dencker[14] equations, however, demonstrated a mean increase of 1.2 Z Scores. This was accompanied by a large SD (1.16) which consequently resulted in 15 children (22%) falling above the upper limit of normal (>1.96 Z Scores). The increase in F_{res} Z Score and decrease in X_5 Z Scores could have significant clinical implications (i.e. over-diagnosis of abnormalities) if interpretation were directly dependent on these equations and limits of normality.

4.7 Interpretation of IOS data in healthy Black children

Differences in IOS were observed when applying reference data based on White children[41] to results obtained from Black children. Furthermore, the limits of normality (defined as +/-1.96 SD) were not applicable in this population due to the variable SD observed. Interpretation of IOS data in Black children was therefore limited. Alternative interpretation strategies include adjusted limits of normality (based on the mean +/-1.96 SD of the values observed in healthy Black children), adjusted Z Scores (i.e. use of an ethnic adjustment factor to re-set the mean Z Scores to zero), or regression analysis to determine the relationship between IOS outcomes and independent variables (such as height, weight, age and sex). The appropriateness and limitations of these interpretative strategies are described in the following sections.

4.7.1 Adjusted limits of normality

In a healthy, normally distributed population the mean (SD) is 0.0 (1.0) and the 95% limits of normality are defined as the mean +/-1.96 SD. In the measured sample of healthy Black children, however, the mean was significantly different from zero, and the SD around the mean was <1.0 in resistance measurements and >1.0 in F_{res} and X_5. The 95% limits of agreement based on these healthy Black children were therefore adjusted to take into consideration the different mean and SD observed for each outcome (Table 4-3).

Table 4-3: Adjusted limits of normality for IOS data.

	Mean (SD)	LLN (95% CI)	ULN (95% CI)
R_5 Z Score	0.63 (0.94)	-1.21 (**-1.44;** -1.02)	2.47 (2.25; **2.70**)
R_{10} Z Score	0.63 (0.75)	-0.84 (**-1.02;** -0.661)	2.1 (1.92; **2.28**)
R_{15} Z Score	0.54 (0.64)	-0.71 (**-0.87;** -0.56)	1.79 (1.64; **1.95**)
R_{20} Z Score	0.59 (0.63)	-0.65 (**-0.79;** -0.50)	1.82 (1.68; **1.97**)
F_{res} Z Score	1.23 (1.16)	-1.04 (**-1.32;** -0.77)	3.50 (3.23; **3.78**)
X_5 Z Score	-0.81 (1.12)	-2.99 (**-3.24;** -2.73)	1.41 (1.15; **1.66**)

LLN= lower limit of normal; ULN = upper limit of normal (calculated as: mean +/-1.96SD). Results expressed as Z Scores. [41] Based on 68 healthy Black children aged 6 to 11 years. R95% confidence intervals around the LLN and ULN were calculated from the standard error of the mean (SEM) and provide a more precise estimation of the limits of normality. Values in bold indicate the most conservative limits, that take the actual sample size of this study into account.

Table 4-3 suggests *conservative* limits of agreement that could be used to interpret IOS results in Black children. The limits of normality differ significantly from the conventional +/-1.96 Z Scores and therefore limit the clinical applicability of these thresholds (i.e. added complication to interpretation if different limits of normality are applied for each outcome). The small sample size (n=68) from which the numbers were derived also limited this interpretative strategy.

4.7.2 Adjusted Z Scores

Black-specific IOS reference equations have not been developed, yet ethnic differences in lung function outcomes have been identified,[8] and the studied population of healthy Black children demonstrated a significant difference from zero Z Scores based on a White population. An interim solution for interpreting IOS data obtained in Black children, may be to apply an ethnic adjustment factor to the measured results prior to applying the reference equations by Dencker.[41] Table 4-4 identifies the calculated "adjustment" factors which were applied (i.e. the measured value was divided by 1.16 for R_5 and R_{15}; 1.17 for R_{10}; 1.18 for R_{20}; 1.28 for X_5 and 1.30 for F_{res}) and hence enabled the group of healthy Black children to be "re-set" to zero Z Scores. Limits of normality (+/-1.96SD) were also recalculated based on the observed SD in the measured sample of children (Figure 4-7).

Table 4-4: Ethnic adjustment factors and calculated limits of normality for IOS.

	Adjustment	LLN (95%CI)	ULN (95%CI)
R_5 Z Score	1.16	-1.6 (**-1.8**; -1.4)	1.6 (1.4; **1.8**)
R_{10} Z Score	1.17	-1.3 (**-1.4**; -1.1)	1.2 (1.1; **1.4**)
R_{15} Z Score	1.16	-1.1 (**-1.2**; -1.0)	1.1 (1.0; **1.2**)
R_{20} Z Score	1.18	-1.1 (**-1.2**; -0.9)	1.1 (0.9; **1.2**)
F_{res} Z Score	1.28	-1.8 (**-2.0**; -1.6)	1.8 (1.6; **2.0**)
X_5 Z Score	1.30	-1.6 (**-1.8**; -1.4)	1.6 (1.4; **1.8**)

LLN= lower limit of normal; ULN = upper limit of normal.

Results calculated from 68 healthy Black children aged 6 to 11 years, based on Dencker reference equations.[41] Limits of normality = mean +/-1.96 SD. 95% confidence intervals around the LLN and ULN were calculated from the standard error of the mean (SEM) and provide a more precise estimation of the limits of normality. Values in bold indicate the most conservative limits, that take the actual sample size of this study into account.

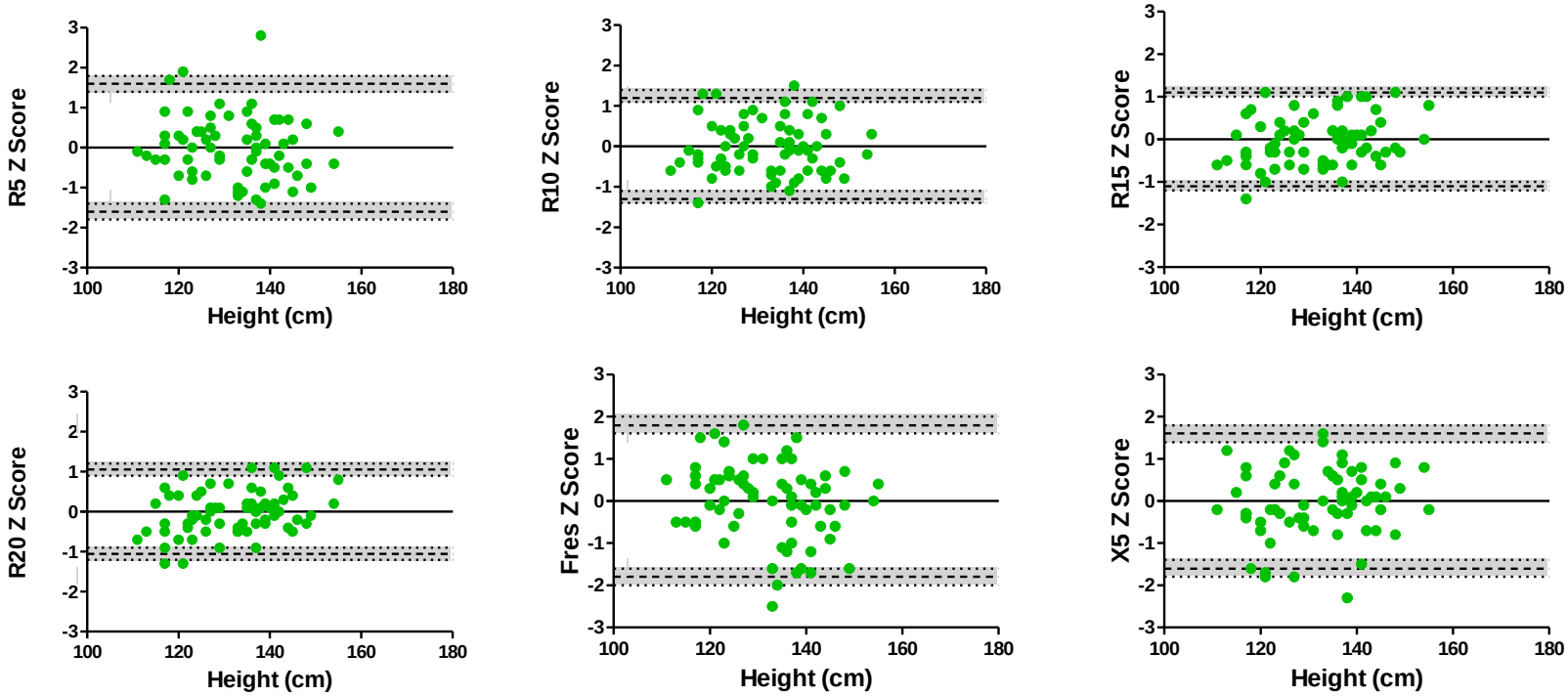

Figure 4-7: IOS outcomes and calculated limits of normality.
Results expressed as Z Scores according to Dencker[41] equations (with an ethnic adjustment) in healthy Black children.
Legend: Adjusted 95% limits of normality calculated as +/-1.96 SD are indicated by the dashed lines, grey shaded area indicates the 95%CI around the limits of normality.
As can be seen, after adjustment 95% of healthy Black children fell within the 95% limits of agreement.

4.7.3 Regression analysis on IOS outcomes

Univariable linear regression analysis was performed to determine the relationship between independent variables and each IOS outcome in healthy Black children aged 4 to 11 years (Table 4-5).

Table 4-5: Univariable analysis on all IOS outcomes independent variables.

	Height	Age	Sex	Weight	BMI
R_5 (kPa·L^{-1}.s)	R^2=0.17***	R^2=0.19***	R^2=0.00	R^2=0.12**	R^2=0.06*
R_{10} (kPa·L^{-1}.s)	R^2=0.21***	R^2=0.22***	R^2=0.00	R^2=0.17**	R^2=0.09*
R_{15} (kPa·L^{-1}.s)	R^2=0.22***	R^2=0.23***	R^2=0.00	R^2=0.19**	R^2=0.11*
R_{20} (kPa·L^{-1}.s)	R^2=0.18**	R^2=0.22*	R^2=0.00	R^2=0.20	R^2=0.04
X_5 (kPa·L^{-1}.s)	R^2=0.18***	R^2=0.07*	R^2=0.00	R^2=0.18*	R^2=0.02
F_{res} (Hz)	R^2=0.01	R^2=0.00	R^2=0.00	R^2=0.04	R^2=0.02
Fdr_{5-20} (kPa·L^{-1}.s)	R^2=0.06***	R^2=0.07***	R^2=0.02	R^2=0.04	R^2=0.02
AX (kPa·L^{-1})	R^2=0.08***	R^2=0.05	R^2=0.01	R^2=0.05	R^2=0.02

*p<0.05. **p<0.01; ***p<0.001

Sex did not account for any variability in any of the IOS outcomes and BMI only contributed a small amount to the variability of R_5, R_{10} and R_{15}; these independent variables were therefore removed from further analysis. No relationship between the independent variables and F_{res} was observed, therefore this outcome was not evaluated further.

Multiple regression analyses were then performed using height, age and weight to determine which combination of independent variables accounted for the most variability in each IOS outcome. Table 4-6 demonstrates the process undertaken using R_5 as an example.

Table 4-6: Combination of regression models describing the association between R_5 and height, age and weight in 68 healthy Black children aged 4-11 years.

Model	Constant	Height (cm)	Age (years)	Weight (kg)	Adjusted R^2
1	1.620	-0.004 (0.003)	-0.035 (0.022)		0.18
2	1.795	-0.007 (0.004)		-0.001 (0.003)	0.15
3	1.302		-0.044 (0.017)	-0.003 (0.002)	0.18
4	1.501	-0.002 (0.005)	-0.036 (0.023)	-0.001 (0.003)	0.20

Data presented as ß (SE)

R_5 had a significant ($p<0.005$) but weak association ($r=0.45$) with height, age and weight. The relationship was defined as:

R_5 (kPa·L-1.s) = 1.501 + (-0.002*height_cm)+(-0.036*age_years)+(-0.001*weight_kg)

This relationship accounted for 20% of the variability of R_5 (R^2=0.20).

Similar relationships were observed in resistance measured at other frequencies:

R_{10} (kPa·L-1.s) = 1.163 + (-0.001*height_cm)+(-0.028*age_years)+(-0.002*weight_kg)

R_{15} (kPa·L-1.s) = 1.022 + (-0.001*height_cm)+(-0.024*age_years)+(-0.002*weight_kg)

R_{20} (kPa·L-1.s) = 1.005 + (-0.001*height_cm)+(-0.019*age_years)+(-0.001*weight_kg)

However, R^2 for each outcome remained low (0.22 for R_{10}, 0.27 for $R_{15,}$ and 0.18 for R_{20}).

Multiple regression analysis with height, age and weight on the remaining IOS outcomes (X_5, Fdr and AX), demonstrated that the addition of age or weight did not significantly contribute to the model. Height was chosen in preference to age to prevent any bias due to restricted growth. The relationships were defined as:

X_5 (kPa·L^{-1}.s) =-0.838 + 0.004*height_cm (R^2 = 0.18)

Fdr_{5-20} (kPa·L^{-1}.s) = 0.626+ -0.003*height_cm (R^2 = 0.06)

AX (kPa·L^{-1}) = 7.06 + -0.036*height_cm (R^2 = 0.08)

R^2 was again very low for these outcomes meaning that these relationships only partly explain the variability seen in IOS outcomes.

4.8 Recommendations for interpreting IOS data in Black children

IOS reference data specifically for Black children were not available, hence attempts to apply ethnic adjustment factors and adjusted limits of normality to interpret R_5, R_{10}, R_{15} R_{20}, X_5 and F_{res} in Black children were made (reference data for AX and Fdr were not available). The limits of normality varied widely for each outcome and potentially obscured real physiological ethnic differences, which could not be identified on the relatively small sample size (n=68). In addition, the varying SD observed for each outcome (which differed from the expected SD of 1) may have skewed the limits of normality. Adjusted Z Scores may simplify interpretation when ethnic differences vary according to each outcome, however the definitive adjustment factor cannot be based on the relatively small sample size. These approaches to interpretation are therefore limited and should be used with caution.

Regression analysis was also used to determine the relationship between each IOS outcome and the relevant determinants (e.g. height, weight, and age), but weak associations were observed and the independent variables only partially accounted for the variability seen with each IOS outcome. In addition, the small sample size prevented new reference equations with limits of normality being developed. Until adequate ethnic-specific reference equations are developed, the most appropriate method of interpreting IOS data in Black children is to make direct comparisons of Z Scores derived from Dencker equations[41] between health and disease. By using Z Scores an adjustment for height and weight, known to differ between health and disease,[136] can be made; however limits of normality were shown to be unreliable in the studied population. Although abnormalities in a given individual cannot be detected using this method, group differences between health and disease can be established. Furthermore, multiple regression analysis can be applied to identify the impact of disease after adjusting for any other relevent determinants. Neither approach identifies individual deficits with confidence.

4.9 Quality control and other factors which may influence IOS

The previous sections reviewed IOS reference equations and concluded with some preliminary recommendations for interpreting IOS measurements in Black children. Prior to applying these interpretative methods in children with SCD, quality control and other factors which potentially influence the variability of IOS measurements in children were evaluated.

4.9.1 Reporting of results

There are a variety of methods available with regards to reporting lung function results, such as expressing the mean/median of several measurements. The extent to which the method of summarising data influenced reported values was examined by performing within-subject comparisons of IOS data from 68 healthy Black children and 59 children with SCD (demographics of these children can be seen in Table 4-11, section 4.10). No statistical differences ($p<0.0001$) between reporting IOS outcomes as mean or median of three measures were observed (Table 4-7).

Table 4-7: Comparison of different methods of reporting IOS outcomes.

	Mean	Median	Mean Diff (95% CI)
R_5 (kPa·L^{-1}.s)	0.87	0.87	0.0 (-0.01; 0.01)
R_{10} (kPa·L^{-1}.s)	0.73	0.73	0.0 (0.00; 0.00)
R_{15} (kPa·L^{-1}.s)	0.67	0.68	0.0 (0.00; 0.00)
R_{20} (kPa·L^{-1}.s)	0.64	0.65	0.00 (-0.01; 0.00)
F_{res} (Hz)	22.0	22.1	-0.09 (-0.36; 0.17)
X_5 (kPa·L^{-1}.s)	-0.31;	-0.30	0.0 (-0.01; 0.00)
AX (kPa·L^{-1})	2.38	2.36	0.01 (-0.02; 0.04)

Results based on 68 healthy Black children and 59 children with SCD aged 6 to 11 years.

4.9.2 Within-test repeatability in health and SCD

Within-test repeatability was compared between 68 healthy Black children and 59 children with SCD reported previously (Table 4-11). The within-test standard deviation (SD) and coefficient of variation (CV) for each outcome were evaluated (Table 4-8). With the exception of X_5 and AX, SD and CV were relatively low for all outcomes, with no clinically relevant differences in within-test repeatability between health and SCD (Table 4-8). The greatest within-test variability was seen in the reactance measurements with X_5 having a within-test CV of ~20%. The most repeatable outcome measure was R_{10} which had a within-test CV of ~5%.

Table 4-8: Comparison of IOS within-test repeatability.

	Standard Deviation			Coefficient of Variation (%)		
	SCD	Health	Mean Diff (95% CI) (SCD- Health)	SCD	Health	Mean Diff (95% CI) (SCD-Health)
R_5 (kPa·L^{-1}.s)	0.08 (0.05)	0.06 (0.04)	**0.02 (0.01; 0.09)****	8.3 (4.7)	6.6 (3.6)	**1.7 (0.3; 3.2)***
R_{10} (kPa·L^{-1}.s)	0.04 (0.03)	0.04 (0.02)	0.0 (0.00; 0.01)	5.7 (3.9)	5.1 (3.5)	0.6 (-0.3; 1.6)
R15 (kPa·L^{-1}.s)	0.04 (0.03)	0.03 (0.02)	0.01 (0.00; 0.02)	6.5 (4.6)	5.0 (3.6)	**1.5 (0.0; 3.0)***
R_{20} (kPa·L^{-1}.s)	0.04 (0.03)	0.03 (0.02)	**0.01 (0.00; 0.02)***	7.6 (4.8)	5.5 (3.3)	2.1 (0.0; 4.6)
F_{res} (Hz)	1.28 (1.76)	1.78 (1.89)	-0.50 (-1.15; 0.16)	6.2 (10.8)	8.6 (10.5)	-2.4 (-6.2; 1.3)
X_5 (kPa·L^{-1}.s)	0.08 (0.06)	0.06 (0.04)	0.02 (-0.06; 0.61)	19.6 (17.8)	19.8 (14.9)	-0.2 (-5.6; 6.2)
AX(kPa·L^{-1})	0.46 (0.35)	0.40 (0.31)	0.06 (-0.06; 0.18)	12.1 (7.8)	18.7 (12.2)	-1.8 (-10.3; 3.0)

Unless stated otherwise, results presented as mean (SD) *p<0.05, **p<0.01

Results based on 68 healthy Black children and 59 children with SCD

4.9.3 Over-read scores

All results reported in this chapter were based on data that had undergone quality control (QC) analysis. The QC criteria were based on current literature[1,39,124] and on consultation with Dr Michael Goldman (leading expert at the time of analysis).[38,124] An "acceptable" IOS measurement was "scored" against several categories and given a score of 2 if the criterion was met; one if partially met and zero if it failed to meet that criterion.

The acceptability categories were as follows:

1) **Stable tidal breathing without drift** (1 point if small amount of drift)
2) **No evidence of notching (air-flow leak)** (1 point if small amount of leak)
3) **Breathing Frequency within 15-25 bpm** (1 point if 25-35 bpm or 10-15 bpm)
4) **Tidal Volume within 400-700 mL** (1 point if 700-850 mL/250-400 mL)
5) **Coherence at 10Hz >0.9** (1 point if 0.7-0.9)
6) **Coherence at 5Hz >0.7** (1 point if 0.5-0.7)
7) **Reactance curves and resistance curves physiologically acceptable shape (no inflexion of curves)** (1 point if one curve slight abnormality)
8) **Data acquisition greater than 20 sec and 4 breaths.** (1 point if 12-20s)

A minimum of three and a maximum of eight trials were undertaken, after visual inspection of the results, the best three trials were selected and the above acceptability criteria were applied. The maximum acceptability score was therefore 48 per measurement session (16 per trial). In addition the following repeatability criteria were applied:

1) **3 superimposable AX curves all within 20% of the largest recorded AX** (1 point if 2 within 20%)
2) **3 superimposable R_{10} curves all within 10% of the largest recorded R_{10}** (1 point if 2 within 10%)

NB: If only one trial was deemed acceptable the measurement session was considered a fail (this did not occur in any of the children undertaking IOS measurements). Where only two trials were acceptable, data were included in the overall analysis (i.e. the comparison between health and SCD), but excluded from the total QC analysis.

Sixty-eight healthy Black children and 59 SCD underwent IOS measurements. No child failed the measurements; however five healthy children (7%) and eight children with SCD (14%) only had two acceptable measures and were therefore excluded from the total QC analysis. Thus 189 baseline measurements from 63 healthy Black children and 153 baseline measurements from 51 children with SCD were evaluated (Table 4-9). The majority of children scored maximum points in stable breathing, airflow leak, coherence at 10Hz and "normal shape" indicating that these criteria are fully achievable in this age group. The largest failures occurred in quantifying coherence at 5Hz and breathing frequency where 36% and 21% of children with SCD respectively failed to achieve the recommended range. This was in contrast to healthy children who had relatively few measurements that "failed" in any category (Table 4-9). Despite some "failures" in some categories, no data were excluded due to low QC scores.

Table 4-9: Frequency table of IOS QC over-read scores.

	SCD			Healthy Black children		
	2 points	1 point	0 points	2 points	1 point	0 points
Stable tidal breathing	86.4	12.5	1.0	87.5	12.5	0
No airflow leak	87.7	11.8	0.5	95.5	4.5	0
Breathing frequency	37.3	42.2	**20.5**	54.5	36.6	8.9
Tidal volume	57.5	37.3	5.1	63.4	32.1	4.5
Coherence at 10Hz	83.4	15.3	1.3	84.8	15.2	0
Coherence at 5Hz	23.5	40.4	**36.1**	84.8	15.2	0
Curves: Normal shape	82.4	14.6	3.1	95.5	3.6	0.9
Acquisition	54	42.5	3.6	75.9	23.2	0.9
AX repeatability	60.7	36.3	4	62.4	32.6	5
R10 repeatability	81.4	16.4	2.2	87.9	11.1	1

Results based on 51 children with SCD and 63 healthy Black children aged 6 to 11years

4.9.3.1 Validation of over-read scores

A subset of IOS data from 49 children (22 healthy children and 27 children with SCD with demographics similar to the entire group) were extracted and reverted back to the raw format (i.e. pre editing/QC analysis). A comparison of data pre- and post-QC was performed using paired t-tests. No significant differences in the absolute values or SD occurred after QC analysis; however there was a trend towards a reduction in within-test SD after edits/QC analysis was applied (Table 4-10).

Table 4-10: Within-subject comparisons of raw and QC data.

	Raw data (no edits)	QC data	Mean Diff (raw-QC) (95%CI)
R_5 (kPa·L^{-1}.s)	0.87	0.87	0.00 (-0.02; 0.03)
R_{10} (kPa·L^{-1}.s)	0.72	0.72	0.00 (-0.02; 0.02)
R_{15} (kPa·L^{-1}.s)	0.64	0.64	0.00 (-0.02; 0.01)
AX (kPa·L^{-1}.s)	2.51	2.52	-0.01 (-0.20; 0.19)
R_5 (kPa·L^{-1}.s)SD	0.11	0.08	0.03 (-0.03; 0.07)
R_{10} (kPa·L^{-1}.s)SD	0.08	0.05	0.03 (-0.08; 0.15)
R_{15} (kPa·L^{-1}.s)SD	0.06	0.04	0.02 (-0.05; 0.01)
AX (kPa·L^{-1})SD	0.7	0.6	0.1 (-0.3; 0.6)

4.9.4 Summary of quality control in IOS

Factors which may influence IOS results were examined. Reporting results as the mean or the median of three measures made no significant difference to the final outcome. Of the children assessed, the majority could achieve the current recommendations of at least three measurements.[39] Within-test, within-occasion repeatability was assessed and found to be low, with the most repeatable outcome being R_{10} (with a coefficient of variation of ~5%). Results were reviewed according to QC criteria (derived from Dr Michael Goldman, unpublished work), which demonstrated that the majority of children could achieve these criteria, however the tidal volume criterion (400-700mL) was too high for the young children and should be modified if applied in the future. The impact of applying the QC criteria revealed no significant difference in raw data and edited data, however results may have been biased as all data were obtained by trained physiologists (JK, LW, KL and RB) who endeavoured to obtain data that met the QC criteria and hence online QC (i.e. encouraging the child to adopt the “acceptable” breathing pattern) occurred. Despite the trend towards a slightly decreased within-test SD post QC, the time spent grading each measurement (about 10 minutes per measurement) makes it clinically impractical. Online visual QC and editing is recommended, but a QC grade is not necessary.

4.10 Applications of IOS in SCD

The previous sections have investigated reference data and methods of qualtiy control and defined within-test repeatability. This final section applies these methods and evaulates the use of IOS in 59 children with SCD in comparison to 68 healthy Black children. The children with SCD were significantly shorter and lighter than their healthy peers despite a similar age range (Table 4-11).

Table 4-11: Comparison of 59 children with SCD and 68 healthy Black children.

	SCD	Healthy Black	Mean Diff (SCD-health) (95%CI)
n (% male)	59 (54%)	68 (41%)	
Age (years)	7.5 (1.1)	8.2 (1.5)	-0.7 (-1.3; -0.1)*
Height Z Score	0.1 (1.1)	0.6 (1.0)	-0.5 (-0.9; -0.2)**
Weight Z Score	0.1 (1.1)	0.8 (1.2)	-0.7 (-1.1; -0.3)***
BMI Z Score	-0.6 (1.1)	0.0 (0.9)	-0.6 (-1.0; -0.2)**

Unless stated otherwise, results presented as mean (SD) *p<0.05, **p<0.01
Demographics expressed as Z Scores[131]

Multiple regression analysis was used to determine the impact of SCD after adjusting for any other relevant determinants (e.g. height, weight, age) for each IOS outcome. The relationships are described in (Table 4-12). After adjusting for age and height, having SCD did not have a significant impact on the R_5 and R_{10} values (which reflect resistance in the small airways), however R_{15} and R_{20} (which reflect resistance in the more central airways) were significantly *lower* in the presence of SCD (mean reduction (95%CI): -0.06 (-0.10; -0.02) $kPa \cdot L^{-1}.s$ and -0.09 (-0.13; -0.05) $kPa \cdot L^{-1}.s$ respectively). Multiple regression also revealed X_5 (a measure of energy storage/elastic forces in the small airways) to be significantly more negative (mean reduction (95%CI): -0.09 (-0.14; -0.05) $kPa \cdot L^{-1}.s$) in the presence of SCD, after adjusting for height.

The outcomes affected most by SCD were AX (mean (95% CI) increase of 1.08 (0.59; 1.57) $kPa \cdot L^{-1}$) and Fdr_{5-20} (mean (95%CI) increase of 0.14 (0.09; 0.19) $kPa \cdot L^{-1}.s$). Given AX quantifies changes in low frequency reactance, and Fdr_{5-20} quantifies changes from low to high frequency resistance it is not surprising that subtle changes in reactance/resistance at any frequency can have larger changes in these outcomes. (Table 4-12).

Table 4-12: The relationship between height, weight and age and the impact of SCD on each IOS outcome.

	Regression equation **(height in cm; age in years; weight in kg; SCD: 1=SCD, 0 = health)**	**SCD coefficient** **(95% CI)**
R_5 (kPa·L^{-1}.s)	1.777+ (-0.005*height) + (-0.032*age) + **(0.059*SCD)**	0.059 (0.00; 0.123)*
R_{10} kPa·L^{-1}.s)	0.381+ (-0.004*height) + (-0.020*age) + **(-0.012*SCD)**	-0.012 (-0.059; 0.035)
R_{15} (kPa·L^{-1}.s)	1.175+ (-0.003*height) + (-0.010*age) + (-0.001*weight) + **(-0.059*SCD)**	-0.059 (-0.100; -0.017)**
R_{20} (kPa·L^{-1}.s)	1.137+ (-0.003*height) + (-0.008*age) + **(-0.087*SCD)**	-0.087 (-0.126; -0.048)***
X_5 (kPa·L^{-1}.s)	-1.142 + (0.006*height) + **(-0.094*SCD)**	-0.094 (-0.137; -0.051)***
AX (kPa·L^{-1}.s)	9.730 + (-0.057*height) + **(1.080*SCD)**	1.080 (0.592; 1.568)***
Fdr_{5-20} (kPa·L^{-1}.s)	0.834 +(-0.005*height) + **(0.138*SCD)**	0.138 (0.091; 0.185)***

*p≤0.05, **p<0.01, ***p<0.001

Result based on 68 healthy Black children and 59 children with SCD aged 4 to 11 years.

When results were adjusted for height alone and expressed as Z Scores using the Dencker equations,[41] similar results were found: With the exception of R_{10} Z Scores, independent t-tests showed significant group differences in all IOS outcomes (Table 4-13, Figure 4-8 and Figure 4-9).

Table 4-13: Comparison of IOS outcomes in 59 SCD and 68 healthy Black children.

	SCD	Healthy Black	Mean Difference (SCD-health) (95%CI)
R_5 Z Score	0.99 (0.90)	0.63 (0.94)	0.36 (0.04 ; 0.69)*
R_{10} Z Score	0.48 (0.81)	0.63 (0.75)	-0.15 (-0.42 ; 0.13)
R_{15} Z Score	-0.02 (0.83)	0.54 (0.64)	-0.56 (-0.82 ; -0.31)***
R_{20} Z Score	-0.18 (0.80)	0.59 (0.63)	-0.77 (-1.02; -0.52)***
X_5 Z Score	-2.13 (1.58)	-0.79 (1.06)	-1.3 (-1.81; -0.87)***
F_{res} Z Score	1.58 (0.69)	1.23 (1.16)	0.35 (0.01; 0.69)*
AX	3.78 (1.54)	2.25 (1.43)	1.53 (1.00; 2.04)***
Fdr_{5-20}	0.40 (0.14)	0.22 (0.13)	0.18 (0.13; 0.22)***

Unless stated otherwise, results presented as mean (SD) *p≤0.05, ***p<0.001
Results expressed as Z Scores,[41] and based on 59 children with SCD and 689 healthy Black children.

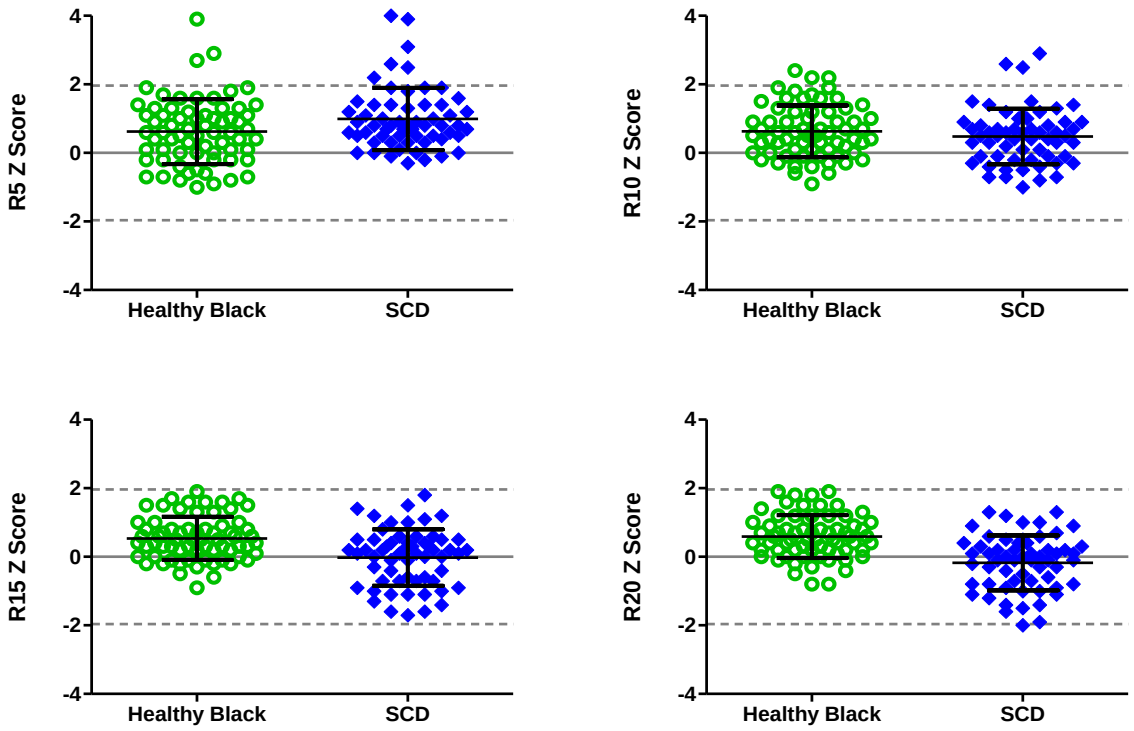

Figure 4-8: Comparison of resistance measured at 5, 10 15 and 20Hz
Results based on 68 healthy Black children and 59 children with SCD**.**
Legend: Results expressed as Z Scores.[41] Black lines denote mean +/-SD for the studied population. Solid and dashed grey lines indicate the mean and limits of normality (+/-1.96 SD) for the reference data.
R_5 (a measure of resistance in small airways) was significantly elevated in SCD; there was no difference in R_{10} between health and SCD and R_{15} and R_{20} (measures of resistance in the larger/central airways) were significantly lower in the presence of SCD.

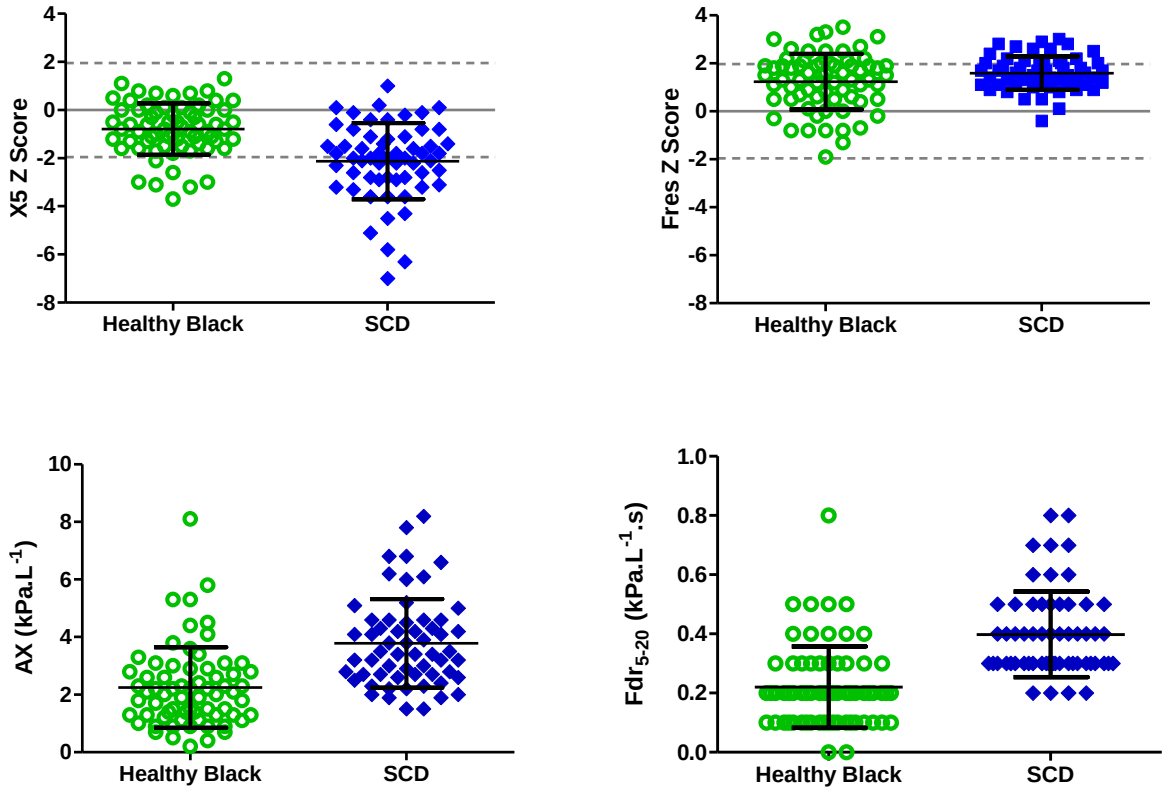

Figure 4-9: Comparison of X_5, F_{res}, AX Z Scores and Fdr_{5-20}.
Results based on 68 healthy Black children and 59 children with SCD aged 4 to 11 years.
Legend: Black lines denote mean +/-SD for the studied population. Solid and dashed grey lines indicate the mean and limits of normality (+/-1.96SD) for the reference data (not available for AX or Fdr).
Significant differences in all outcomes were observed. The reduced X_5 and increased F_{res} in SCD both contribute to the elevated AX observed in SCD which reflects changes in the periphery. The elevated Fdr_{5-20} reflects changes in both the periphery (increased R_5) and the central airways (reduced R_{20}).

R_5 Z Score was significantly increased in SCD compared with the healthy children. In addition, when reviewing the relative changes in resistance measured at low frequencies (which represent the small airways), to resistance measured at high frequencies (which represent the central airways) a frequency dependence was observed in the SCD group. Resistance in the healthy children however, remained relatively stable regardless of the frequency at which it was measured (as expected), albeit with a larger spread around the mean (Figure 4-10). This frequency dependence of resistance was also highlighted by the t-tests on Fdr which revealed children with SCD to have significantly higher Fdr_{5-20} when compared to healthy Black children ($p<0.0005$, mean difference (95% CI): 0.18 $kPa \cdot L^{-1}.s$ (0.13; 0.22)) (Figure 4-9). These results may be indicative of peripheral airway obstruction; however they only became apparent because the SCD group had a mean reduction in resistance at high frequency in comparison to healthy children (mean difference: -0.77 Z Scores) as well as a slight increase in resistance at low frequency (mean difference: +0.4 Z Scores). Such results may suggest the SCD group have reduced resistance within the central airways, and a relative increased resistance in the peripheral airways. However, the large SD around the mean observed in health limits the interpretation of these findings.

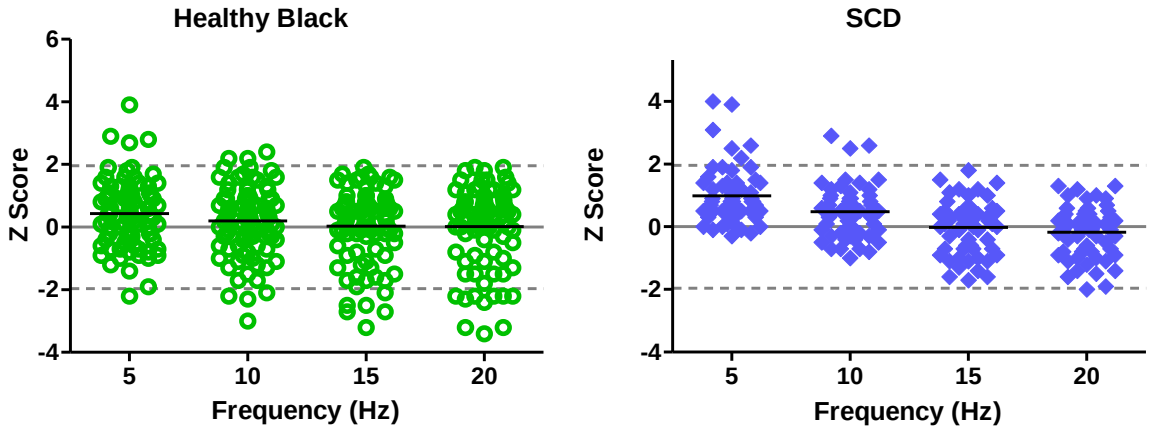

Figure 4-10: Frequency dependence of Resistance in health and SCD
Legend: Left panel (green) = 68 healthy Black children; Right Panel (Blue) = 59 children with SCD. Black lines denote mean for the studied population. Solid and dashed grey lines indicate the mean and limits of normality (+/-1.96SD) for the reference data. [41]
In health, resistance remains relatively stable regardless of the frequency at which is measured. In SCD there is a slight increase in resistance in the small airways (low frequency resistance) and a larger reduction in resistance in the central airways (high frequency resistance), and thus a relative increase in Fdr is observed. However the large spread around the mean observed in health limits the confidence with which these results can be interpreted.

4.10.1 Summary of IOS results in SCD

Fifty-nine children with SCD were compared with 68 healthy Black children of the same age (4 to 11 years). Interpretation on an individual basis was restricted due to the lack of adequate reference data and limits of normality, however multiple regression analysis revealed SCD had a significant impact on high frequency resistance (R_{15} and R_{20}), X_5, AX and Fdr after adjusting for height, age and weight (where applicable). These statistically significant differences were small (0.09 $kPa \cdot L^{-1}.s$ in R_{20}), equating to less than one SD per outcome. Similarly, direct comparisons of health and SCD when results were expressed as Z Scores revealed significant group differences. R_5 was slightly elevated in SCD, whereas R_{15} and R_{20} were significantly reduced.

Results suggest a decreased resistance in the more central airways relative to the periphery in SCD. This may suggest peripheral airway obstruction, and/or reflect increased traction and hence increased airway calibre in the central airways in the presence of restrictive lung disease. Further interpretation of these results is only possible in the light of other lung function assessments and clinical symptoms. The clinical usefulness of IOS in combination with other lung function tests and respiratory symptoms will be examined further in Chapter 8.

4.11 Repeatability and the bronchodilator response

The previous section investigated the applications and interpretation of IOS in distinguishing between health and disease. In addition to identifying group differences in baseline IOS measurements, IOS has been proposed as a useful outcome measure in the assessment of bronchodilator response (BDR).[37,72,73,135] Appropriate interpretation of the BDR first requires knowledge of the between-test, within-occasion repeatability in health and disease, and whether or not healthy children exhibit a BDR. The following section will first review the between-test repeatability in health and in SCD, then determine the thresholds for defining a significant BDR before finally assessing the clinical role IOS plays in assessing BDR in children with SCD. Outcome measures analysed included R_5, R_{10}, R_{20} AX and Fdr_{5-20}.

4.11.1 Between test repeatability in health and SCD

Twenty-two healthy Black children (mean (SD) age: 10.1 (2.1) years) and 27 children with SCD (mean (SD) age: 10.4 (2.6) years) underwent baseline measures of IOS and repeated measures after a 15 minute interval with no intervention. Paired t-tests revealed no significant difference between the two measurement sessions, and the between-test repeatability was the same in all outcomes in both health (Table 4-14) and SCD (Table 4-14), although between-test repeatability in AX was far greater (but not statistically significant) in SCD.

Table 4-14: Between test repeatability and limits of agreement of IOS in health.

	Baseline (A)	Repeated (B)	Mean Diff (B-A) (95% CI)	95% Limits of Agreement	
				Lower limit (95% CI)	**Upper limit (95% CI)**
R_5 (kPa·L^{-1}.s)	0.72 (0.15)	0.72 (0.15)	0.00 (-0.03; 0.04)	-0.15 (**-0.19**; -0.12)	0.16 (0.13; **0.19**)
R_{10} (kPa·L^{-1}.s)	0.61 (0.12)	0.62 (0.13)	0.01 (-0.02; 0.04)	-0.12 (**-0.15**; -0.09)	0.14 (0.11; **0.17**)
R_{20} (kPa·L^{-1}.s)	0.57 (0.12)	0.58 (0.12)	0.01 (-0.02; 0.04)	-0.11 (**-0.14**; -0.09)	0.10 (0.07; **0.12**)
Fdr_{5-20} (kPa·L^{-1}.s)	-0.14 (0.06)	-0.14 (0.05)	0.00 (-0.01; 0.01)	-0.11 (**-0.14**; -0.09)	0.10 (0.08; **0.12**)
AX (kPa·L^{-1})	1.50 (0.76)	1.43 (0.85)	-0.07 (-0.32; 0.19)	-1.19 (**-1.38**; -0.99)	1.04 (0.85; **1.24**)

Unless stated otherwise, results presented as mean (SD)
Results based on 22 healthy Black children aged 6 to 11 years.
95% confidence intervals around the LLN and ULN were calculated from the standard error of the mean (SEM) and provide a more precise estimation of the limits of normality. Values in bold indicate the most conservative limits, that take the actual sample size of this study into account.

Table 4-15: Between test repeatability of IOS in SCD.

	Baseline (A)	Repeated (B)	Mean Diff (B-A) (95% CI)
R_5 (kPa·L^{-1}.s)	0.73 (0.21)	0.70 (0.21)	-0.03 (-0.06; 0.00)
R_{10} (kPa·L^{-1}.s)	0.63 (0.16)	0.60 (0.17)	-0.03 (-0.05; 0.00)
R_{20} (kPa·L^{-1}.s)	0.58 (0.13)	0.55 (0.14)	-0.03 (-0.05; 0.00)
Fdr_{5-20} (kPa·L^{-1}.s)	0.15 (0.02)	0.15 (0.02)	0.00 (-0.02; 0.03)
AX (kPa·L^{-1})	1.81 (1.45)	1.63 (1.41)	-0.18 (-0.36; 0.02)

Unless stated otherwise, results presented as mean (SD). Results based on 25 children with SCD.

4.11.2 Bronchodilator response in health

Twenty-five healthy Black children (mean (SD) age: 8.6 (1.3) years) underwent BDR measurements. Bland and Altman analysis on the between-test repeatability was performed to calculate the 95% limits of agreement from which the thresholds for a significant BDR could be determined (i.e. results which fell outside these limits constituted a significant change from baseline) (Table 4-14). Each IOS outcome post BD was plotted using the limits of repeatability defined in Table 4-14, thus allowing the BDR in health to be examined. All outcomes identified a trend towards a slight BDR in health, with R_5 and R_{10} identifying six children in whom a significant BDR was observed (Figure 4-11). Four of these children also demonstrated a significant reduction in R_{20} (Figure 4-12), whereas AX and Fdr only picked up two of these children (Figure 4-13).

The extent to which BDR occurs in health should be taken into account when assessing BDR in disease. Six healthy children demonstrated a significant BDR when using R_5 and R_{10} as the main outcome measures, and many of the other healthy children demonstrated some, albeit statistically insignificant, bronchodilator responsiveness (seen by the overall trend for a decrease in IOS outcomes post BD) (Figure 4-11, Figure 4-12 and Figure 4-13). Bland and Altman analysis of baseline and post BD measurements in health were performed and the 95% limits of agreement were used as the threshold for determining a positive BDR in children with SCD (Table 4-16).

Table 4-16: 95% Threshold for a bronchodilator response using IOS.

	95% Limits of agreement:	
	Lower limit (95% CI)	**Upper limit (95% CI)**
R_5 (kPa·L^{-1}.s)	-0.47 (**-0.54**; -0.41)	0.18 (0.12; 0.25)
R_{10} (kPa·L^{-1}.s)	-0.30 (**-0.34**; -0.26)	0.09 (0.06; 0.13)
R_{20} (kPa·L^{-1}.s)	-0.25 (**-0.29**; -0.21)	0.13 (0.09; 0.17)
Fdr_{5-20} (kPa·L^{-1}.s)	-0.29 **(-0.33**; -0.25)	0.12 (0.08; 0.16)
AX (kPa.L^{-1})	-3.74 (**-4.28**; -3.20)	1.76 (1.22; 2.30)

Results based on 25 healthy Black children. 95% confidence intervals around the LLN and ULN were calculated from the standard error of the mean (SEM) and provide a more precise estimation of the limits of normality. Values in bold indicate the most conservative threshold for a BDR, that take the actual sample size of this study into account.

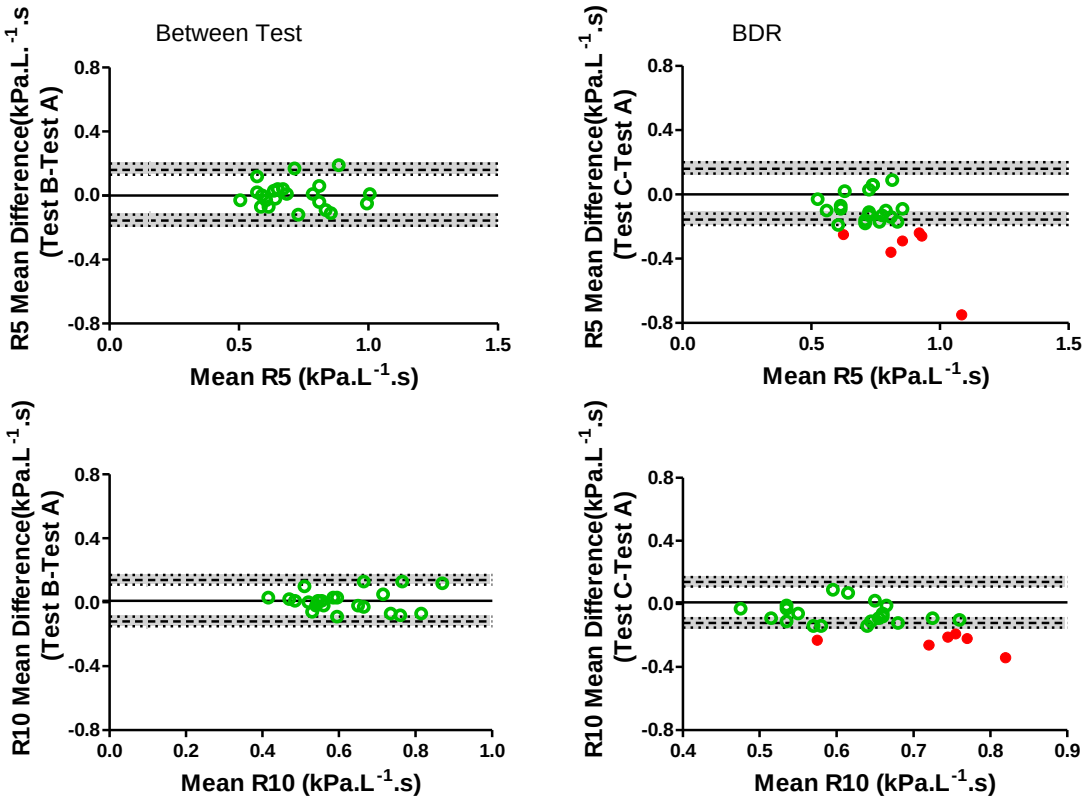

Figure 4-11:Between test repeatability and BDR of R_5 & R_{10} healthy Black children.
Legend: Test A = baseline; Test B = repeatability; Test C = post bronchodilator. Dashed lines denote 95% limits of agreement calculated from the between test repeatability (grey shaded area denotes the 95%CI around these limits). Red dots indicate those children in whom a significant BDR was observed (i.e. results below the lower limit of repeatability). Six healthy children were identified as a having a significant BDR

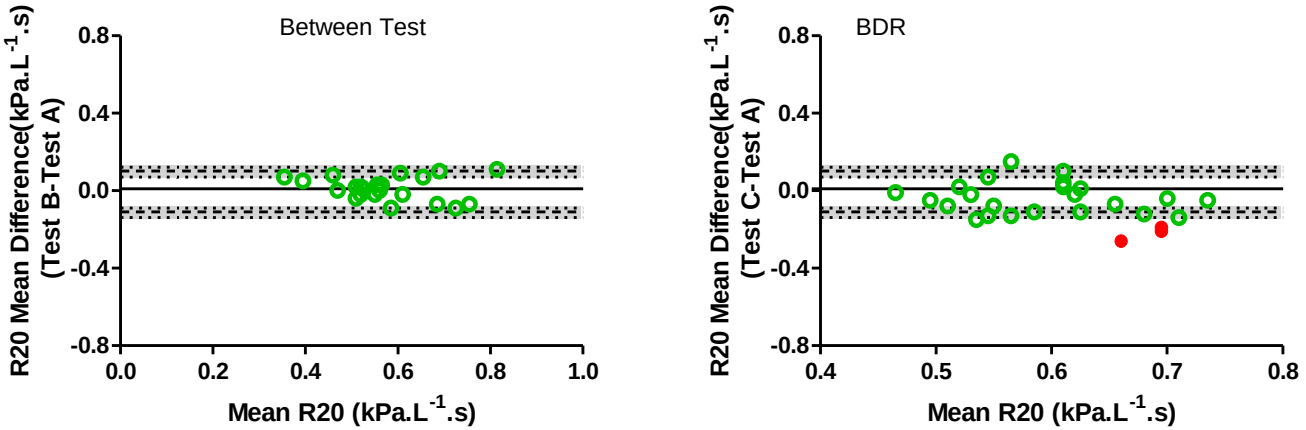

Figure 4-12: R_{20} between test repeatability and BDR healthy Black children.
Legend: Test A = baseline; Test B = repeatability; Test C = post bronchodilator. Dashed lines denote 95% limits of agreement calculated from the between test repeatability (grey shaded area denotes the 95%CI around these limits). Red dots indicate those children in whom a significant BDR was observed (i.e. results below the lower limit of repeatability).
R_{20} only identified a significant BDR in 3 healthy children (compared to the 6 that were identified by R_5 and R_{10}).

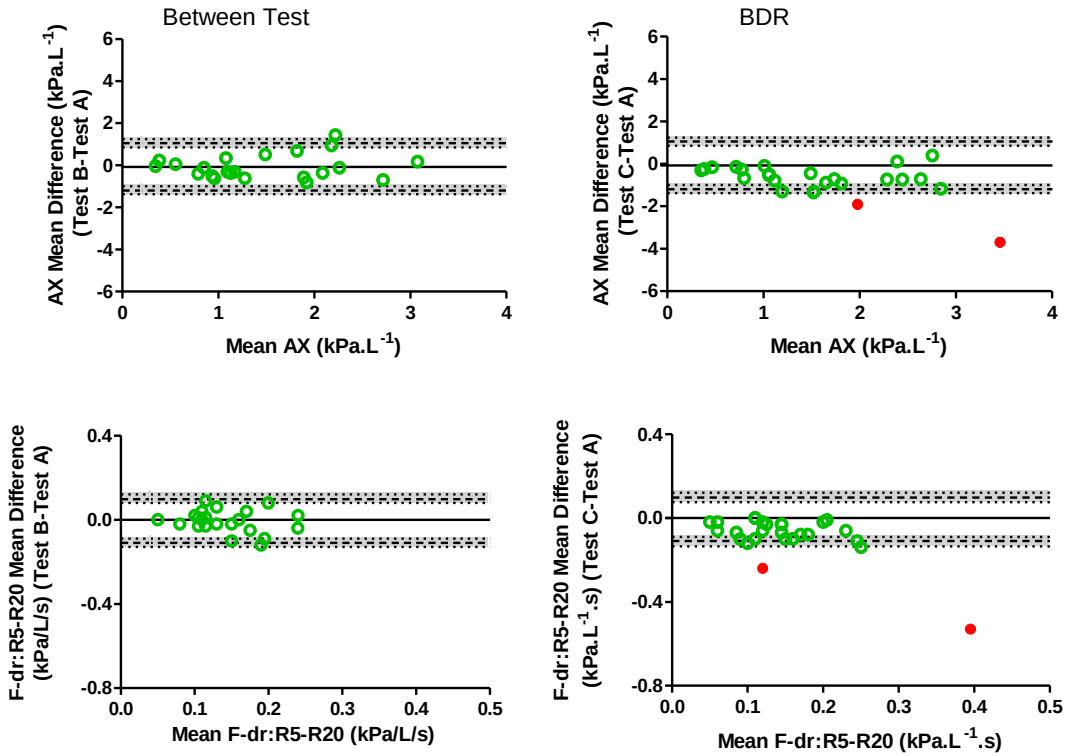

Figure 4-13: AX and Fdr_{5-20} between test repeatability and BDR in healthy Black children
Legend: Test A = baseline; Test B = repeatability; Test C = post bronchodilator. Dashed lines denote 95% limits of agreement calculated from the between test repeatability (grey shaded area denotes the 95%CI around these limits). Red dots indicate those children in whom a significant BDR was observed (i.e. results below the lower limit of repeatability). The same two children were identified as having a significant BDR with these outcomes, however, an additional 4 children were identified by R_5 and R_{10}.

4.11.3 Bronchodilator response in SCD

Fifty-four children with SCD (mean (SD) age: 7.6 (2.0) years) underwent baseline and BDR assessment using IOS measurements. With the exception of R_{20} in SCD, a statistically significant BDR was observed in all outcomes in both healthy children and children with SCD (Table 4-17).

A clinically significant BDR, however, is a change over and above that seen in healthy children of the same age. The thresholds for a significant BDR for each IOS outcome were derived from Bland and Altman analysis on baseline and BDR measures in health (Table 4-16). The BDR in SCD was plotted next to the BDR in health to examine the extent to which BDR occurred in SCD. There were no significant differences between the BDR in health and SCD when R_5 (Figure 4-14), R_{10} and R_{20} were the outcome measures (Figure 4-15). Fdr_{5-20} identified five children with SCD with a significant BDR (over and above that seen in health). There was a significant difference between the absolute change post BD seen in SCD and health (mean difference (SCD-health): -0.08 $kPa \cdot L^{-1}.s$ ($p<0.005$, 95%CI: -0.13; -0.03) (Figure 4-16). AX only identified one child with SCD with a clinically significant BDR; however the mean difference in BDR in health and SCD was not significant (Figure 4-16).

Table 4-17: Comparison of baseline and post bronchodilator results in 25 healthy Black children and 59 children with SCD aged 4 to 11 years.

	Healthy Black			SCD		
	Baseline	**Post**	**Mean diff (95% CI)**	**Baseline**	**Post**	**Mean diff (95% CI)**
R_5 (kPa·L^{-1}.s)	0.82 (0.19)	0.68 (0.10)	-0.14 (-0.21;-0.08)***	0.97(0.22)	0.84 (0.20)	-0.13 (-0.17; -0.10)***
R_{10} (kPa·L^{-1}.s)	0.69 (0.12)	0.59 (0.08)	-0.10 (-0.06; -0.01)***	0.76 (0.15)	0.68 (0.15)	-0.08 (-0.11; -0.06)***
R_{20} (kPa·L^{-1}.s)	0.63 (0.10)	0.57 (0.07)	-0.06 (-0.10; -0.02)**	0.65 (0.12)	0.68 (0.15)	0.03 (0.00; 0.06)
Fdr_{5-20} (kPa·L^{-1}.s)	0.19 (0.11)	0.11 (0.06)	-0.08 (-0.13; -0.02)**	0.33 (0.13)	0.17 (0.07)	-0.16 (-0.20; -0.14)***
AX (kPa.L^{-1})	2.19 (1.60)	1.20 (0.73)	-0.99 (-1.56; -0.42)**	3.78 (1.58)	2.71 (1.30)	-1.07 (-1.35; -0.80)***

Mean diff (Post-Baseline); Unless stated otherwise, results presented as mean (SD) *p<0.05, **p<0.01, ***p<0.001

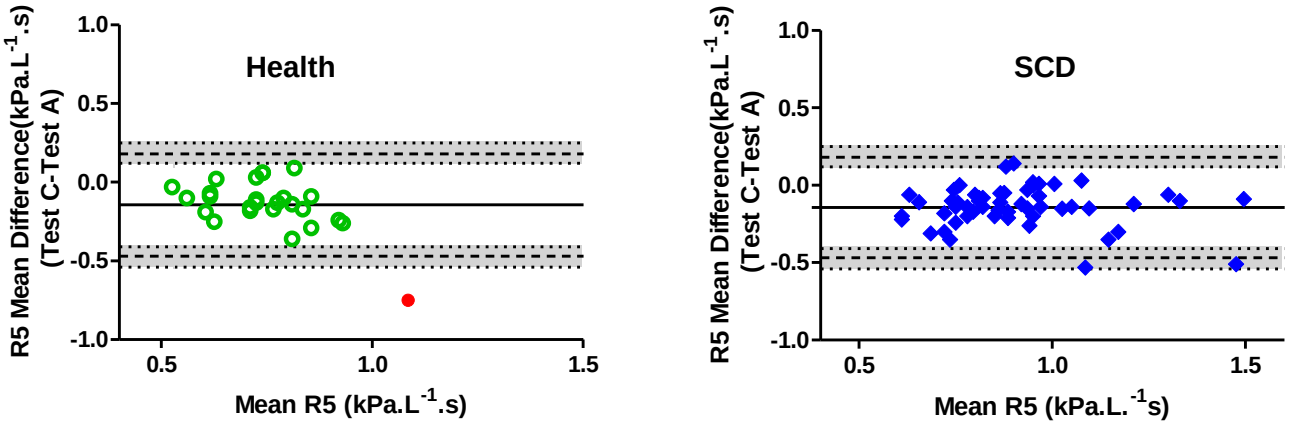

Figure 4-14: BDR measured by R_5 in health and in SCD
Legend: Dashed lines denote 95% limits of agreement calculated from the bronchodilator response observed in health (grey shaded area denotes the 95%CI around these limits). Red dots indicate those children in whom a significant BDR was observed (results below the lower limit of normal).

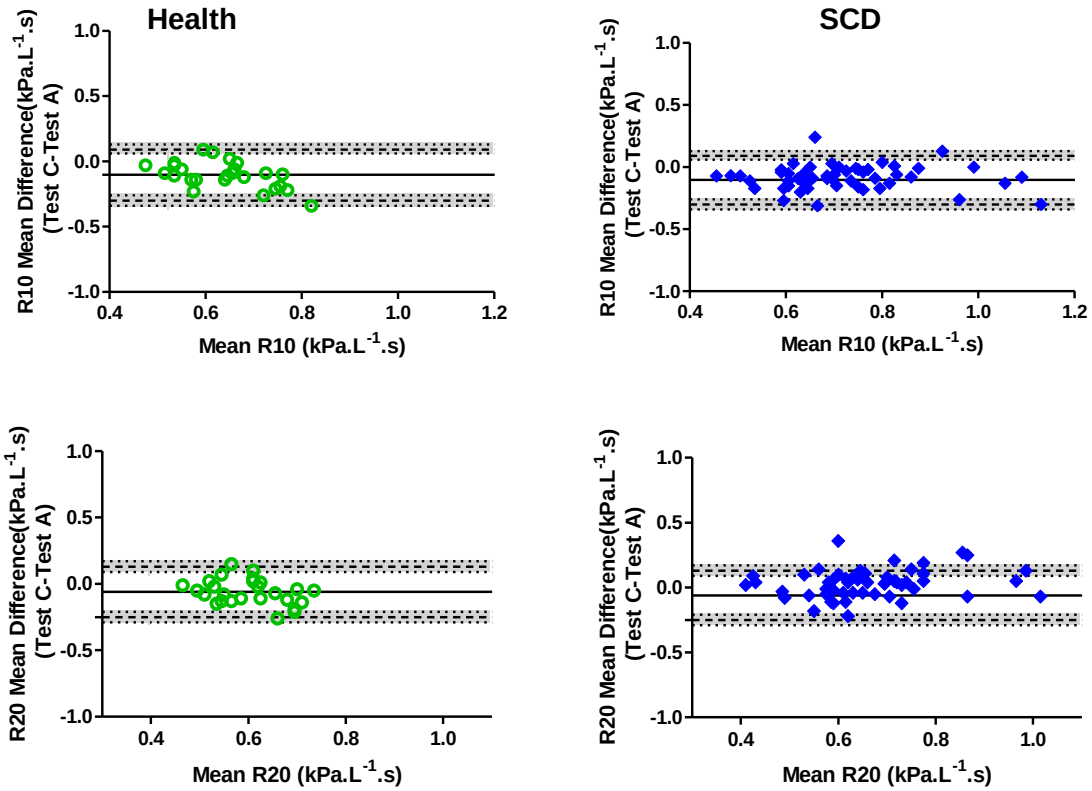

Figure 4-15: BDR in health and in SCD using R_{10} and R_{20} as the outcomes.
Legend: Dashed lines denote 95% limits of agreement calculated from the bronchodilator response observed in health (grey shaded area denotes the 95%CI around these limits).

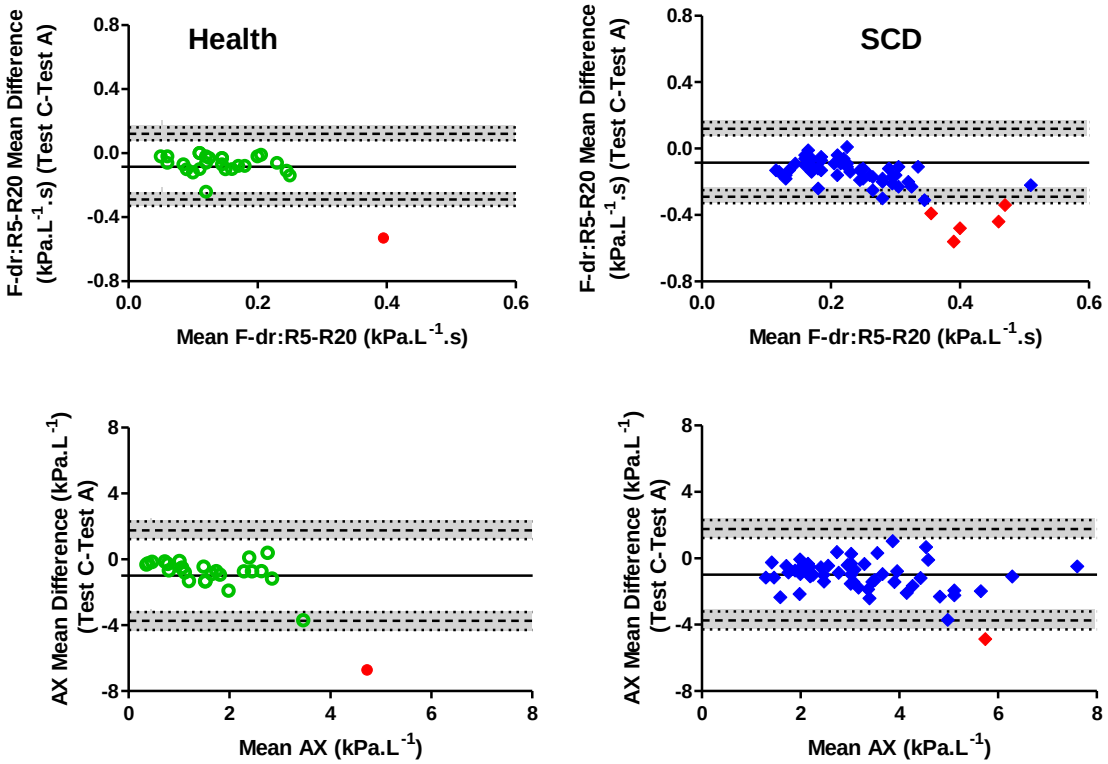

Figure 4-16: BDR in health and in SCD using Fdr_{5-20} and AX as the outcomes.
Legend: Dashed lines denote 95% limits of agreement calculated from the bronchodilator response observed in health (grey shaded area denotes the 95%CI around these limits). Red dots indicate those children in whom a significant BDR was observed (results below the lower limit of normal).

4.11.4 Summary of IOS and the bronchodilator response

Impulse oscillometry was used as an outcome measure in the assessment of BDR in 59 children with SCD. A statistically significant BDR was seen in all outcomes, in both healthy children and children with SCD, however only five children with SCD were identified as having a significant BDR (over and above that seen in health) when using Fdr_{5-20} as the main outcome primarily due to the reduction in R_5 in these children post bronchodilator. Resistance measured at 5Hz and 10Hz in isolation did not identify any clinically important BDR in SCD. The results suggest that as a group, children with SCD do not demonstrate a clinically relevant response to bronchodilator. Those children with some BDR observed in Fdr changes are investigated further in combination with other lung function tests and the clinical status in Chapter 8.

In summary, IOS can be used in the assessment of BDR, however the bronchodilator response observed in health must be taken into account prior to interpreting BDR in lung disease. R_{20} did not identify a clinically relevant change from baseline and should not be used as an outcome measure for assessing BDR. R_5 and R_{10} may be used, but a clinically relevant BDR would be a fall >0.15kPa·L^{-1}.s (15%). These results suggest that Fdr_{5-20} may be the most useful outcome measure in monitoring response to bronchodilator with IOS, as it does not detect many healthy children, but picks up a few children with SCD. The benefit of Fdr_{5-20} as an outcome measure is that it reflects resistance over a range of frequencies and therefore describes the change in resistance across the bronchial tree, rather than at discrete frequencies. The thresholds for Fdr and AX are larger at 0.1kPa·L^{-1}.s (40%) and 1.0kPa·L^{-1} (40%) respectively. Further work with a larger sample size and other disease groups are required to elucidate the appropriate threshold for BDR if this measurement is applied in the future.

4.12 Summary

This chapter has demonstrated discrepancies between the two reference datasets available for IOS in White children and significant differences of +0.5 Z Scores in resistance at all frequencies and -0.8 Z Scores in X_5 were observed in Black children. The differences observed are thought to be due to ethnic differences in lung function. However, the assessment of IOS in White children was not included in the SAC protocol and therefore a direct comparison between White and Black children could not be undertaken and the differences observed in Black children cannot be assumed to be entirely due to ethnicity. Attempts were made to adjust the limits of normality and apply ethnic adjustment factors to enable interpretation in Black children but these were found to be limited due to the sample size upon which they were based.

The clinical usefulness of IOS was assessed by making group comparisons between health and SCD. Multiple regression analysis was used to determine the impact of SCD after adjusting for the relevant determinants, and significant differences between SCD and healthy Black children of the same age were observed. Low frequency resistance (reflecting the small airways) was slightly increased in children with SCD compared to the healthy children however, more significantly, high frequency resistance (reflecting the central airways) was reduced in children with SCD, suggesting lower resistance within the central airways, possibly relating to restrictive lung disease (see Chapter 6: 'Plethysmographic lung volumes').

Finally, IOS was evaluated as an outcome measurement for BDR. The between-test, within-occasion repeatability, and BDR was first established in health, prior to assessing it in SCD. As a group, children with SCD did not exhibit BDR over and above that seen in health. Further work on recommendations and the development of reference equations are required, before IOS can be used clinically.

5 Specific Airways Resistance

5.1 Introduction

Plethysmographic specific airways resistance (sR_{aw}) has proved to be a feasible and useful outcome measure in clinical research studies of preschool children with cystic fibrosis and wheezing disorders.[82,86,87,91,137-139] However its use as a valid outcome measure in clinical management has been limited by the lack of consensus with regards to equipment, measurement conditions, data collection, analytical strategies and reference data. Although sR_{aw} is internally adjusted for differences in lung volume and it is likely that ethnic differences do not exist, this has not been elucidated; thereby limiting interpretation of sR_{aw} in Black children.

This chapter will describe the collation of healthy sR_{aw} data from international centres; the subsequent investigation of methodological differences between these centres and the development of recommendations and reference equations for sR_{aw} measurements in children. These results have been published in the *European Respiratory Journal*.[128] An evaluation of these recommendations in healthy Black children is undertaken to determine if ethnic differences exist. This is followed by an assessment of the potential use of sR_{aw} as an outcome measure in the clinical management of children with Sickle Cell Disease (SCD).

5.2 Aims

The primary aim was to establish appropriate methods for the application and interpretation of sR_{aw} measurements in young children and to develop reference data.

The secondary aims were to:

i. To determine the extent of ethnic differences in sR_{aw} between healthy Black and healthy White children after adjusting for height, sex and age.
ii. To evaluate the extent to which sR_{aw} detects lung disease in children with SCD.

5.3 Objectives

The primary aim was to improve interpretation of sR_{aw} in young children; this was achieved by

i. Collating available reference data for sR_{aw} in young children
ii. Developing reference equations and recommendations for use of sR_{aw} in children
iii. Assessment of the extent to which any bias occurred in results from healthy children when assessed using standardised protocols in different international centres

5.4 Hypothesis

The primary aim here was to collate normative data and generate reference equations; therefore this part of the study was not hypothesis driven. Regarding the application of sR_{aw} in healthy Black children, the hypothesis is that there are no ethnic differences in sR_{aw} between Black and White children.

5.5 Subjects and sample size

The Asthma UK initiative (see Chapter 2, section 2.3.3) provided the majority of the data for this chapter: Plethysmographic sR_{aw} data from healthy children aged 2 to 11 years were collated from five international centres, all of whom had published studies reporting the measurement of sR_{aw} in healthy children.[16,86,104,129,140-142] Healthy Black children were recruited from the SLIC and SAC study and the children with SCD were recruited from the SAC study (see Chapter 2, section 2.3). Age was limited to <10 years since the reference equations were also limited to this age range.

Based on the literature,[16,86,104,129,140-142] the Asthma UK initiative estimated that differences in sR_{aw} of 0.2kPa·s between centres or 0.1k Pa·s within-subject would be clinically or physiologically significant, such differences approximating one standard deviation (SD) for between and within-subject variability respectively.[143] To determine group differences (i.e. differences between Black and White children, or between health and disease) a sample size of 64 children in each group would enable differences of 0.5 SD to be detected with 80% power at the 5% significance level.

5.6 Collation of reference data

The Asthma UK Collaborative Initiative was established in 2005 to develop centile charts and to investigate the impact of sex and age on interpretation of lung function measurements. Further details of data collection and data cleaning can be found in chapter 2 (section 2.6.5) In brief, sR_{aw} data were collected in healthy children aged 2 to 11 years. All participating centres were asked to provide detailed information about recruitment, population characteristics, equipment and measurement protocols.

Five centres contributed 2,872 sets of sR_{aw} data from 2,347 children measured between 1995 and 2008. Population characteristics can be found in Table 5-1.

Table 5-1: Population characteristics in 2,872 healthy children in whom sR_{aw} measurements were collated

	Centre 1	Centre 2	Centre 3	Centre 4	Centre 5
n (% boys)	38 (58%)	40 (50%)	828 (55%)	472 (51%)	1494 (53%)
Age (years)	7.1 (1.1)	4.5 (1.0)	5.1 (1.0)	6.6 (2.1)	4.2 (1.0)
Height Z	0.2 (0.9)	0.8 (1.3)	0.4 (1.1)	0.2 (1.0)	0.2 (1.0)
Weight Z	0.5 (1.4)	0.4 (0.6)	0.2 (1.3)	0.2 (1.1)	0.2 (1.0)
BMI Z	0.5 (1.6)	-0.1 (1.0)	-0.1 (1.0)	0.1 (1.1)	0.1 (1.)
% White:	100%	100%	100%	77%	93%
% Non-White:	-	-	-	3%	6%
% Unknown ethnicity:	-	-	-	20%	1%

Footnote: Anthropometry measurements were expressed as Z Scores according to equations by the CDC.[131]

White subjects of European descent contributed 2531 (88%) of the data points; 93 data points (3%) were recorded as “non-White”, whereas ethnicity was not recorded in 248 (9%) subjects (Table 5-1 and Figure 5-1). The limited data in non-White subjects precluded analysis according to ethnic origin; hence these subjects were excluded from the reference equations (although impact of ethnicity was investigated in section 5.10).

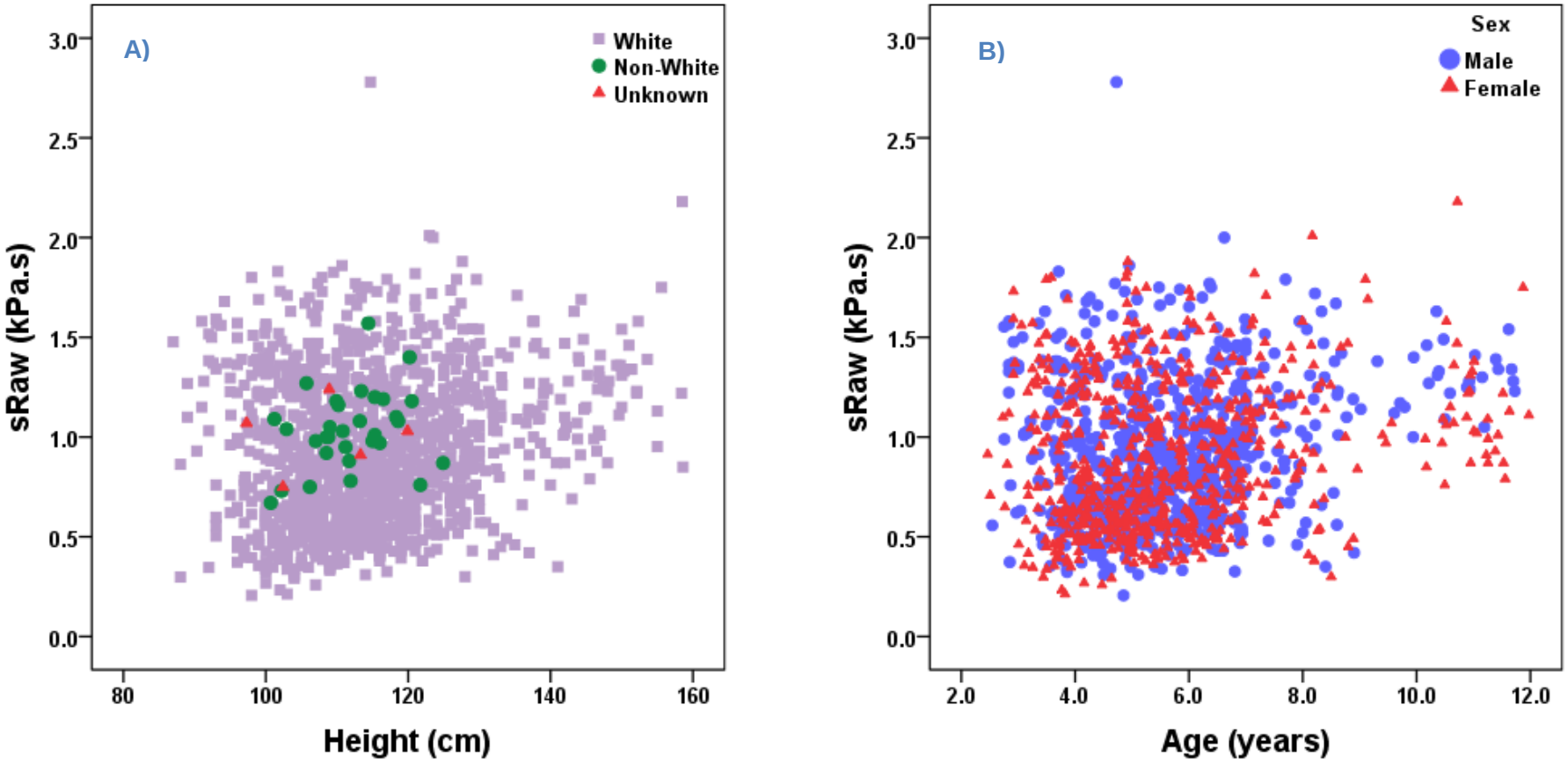

Figure 5-1: sR_{aw} Vs. height (A) and age (B) in children aged 3 to 11 years studied in five international centres
The majority of the data came from White children. The small amount of sR_{aw} data from "non-White" and unknown ethnicities did not appear to differ from the White children (A). Sex differences were not apparent (B)

In total, sR_{aw} data from 2,347 children were evaluated. Individual sR_{aw} values ranged from 0.21 - 2.82kPa·s, with the mean (SD) sR_{aw} from these five centres ranging from 0.55 (0.18) to 1.29 (0.30)kPa·s. One-way ANOVA analysis revealed significant between centre differences ($p<0.0001$) (Figure 5-2).

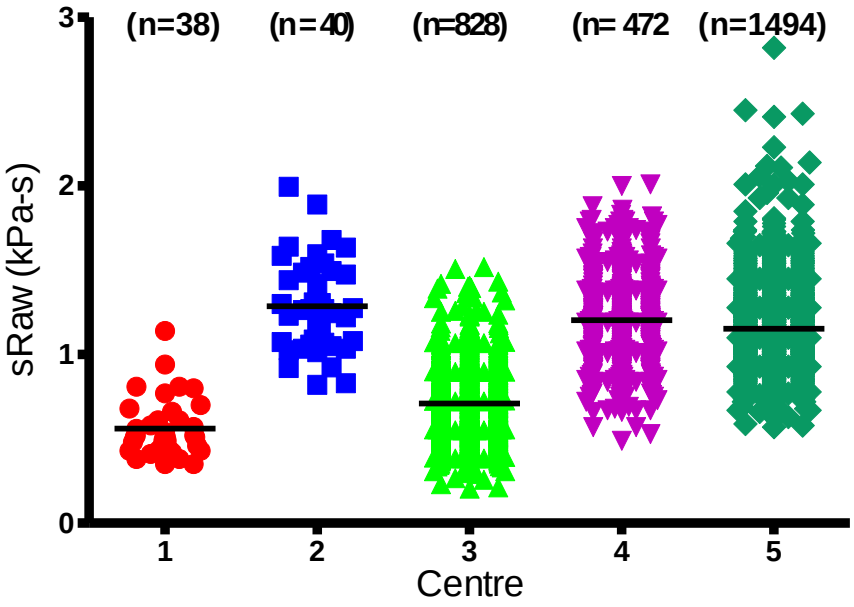

Figure 5-2: sR_{aw} in children aged 3 to 11 years from five international centres.
Significant between centre differences were observed. The difference between the highest and the lowest mean sR_{aw} was 0.74kPa·s.
NB: Two centres (1 & 4) reported sR_{eff} and the remaining centres reported $sR_{tot.}$ A definition of the different sR_{aw} outcomes can be found in the section 5.6.2.1, and the impact of different methods of reporting sR_{aw} investigated in section 5.6.2.3

The significant inter-centre differences prompted an investigation into methodological differences between each centre. Where differences in methodology were observed, sub-analyses were conducted to establish the potential impact of these differences, and resulted in the exclusion of two centres (centres 1 and 3). A final dataset of 1908 sR_{aw} measurements were collated and sR_{aw} reference equations were developed (section 5.7). Further analyses into other factors which may influence sR_{aw} were undertaken before developing recommendations for use (section 5.8.6).

The following section identifies the inter-centre differences and the sub-analyses undertaken which led to the exclusion of two centres, and the collation of data from three centres to generate reference equations.

5.6.1 Inter-centre differences

The Asthma UK initiative collated 2,872 sets of sR_{aw} data from five centres, however the individual sR_{aw} values ranged from 0.21 - 2.82kPa·s and significant inter-centre differences were observed. There was a spread of age across the five collaborating centres (Table 5-1), and one-way ANOVA analysis revealed significant differences in age across the five centres. The impact of age, sex and centre were investigated using regression analysis.

5.6.1.1 Influence of age

Simple linear regression on sR_{aw} and age revealed a strong significant ($p<0.0001$) correlation between the two variables (Figure 5-1B). Nine percent of the variance seen in sR_{aw} could be explained by age ($r^2 = 0.09$), and the relationship was defined as:

$sR_{aw} = 1.179 + -0.021*age\_years$

Thus with every year increase in age, sR_{aw} decreases by 0.02 kPa·s, such that the sR_{aw} predicted at 3, 7 and 10 years is 1.11 kPa·s, 1.03 kPa·s and 0.97 kPa·s respectively. Given the marked between subject variability at any given age, these differences were not physiologically significant, however, age was taken into consideration in the final reference equations (Table 5-5, section 5.7).

5.6.1.2 Regression of age, sex and centre

Multiple regression analysis was used to determine how much of the variance in sR_{aw} could be explained by age alone (section 5.6.1.1) and by age, sex and centre in combination. The coefficient for age, sex and centre was -0.16, -0.06 and 0.59 respectively. Thus, although all three variables made a significant unique contribution to sR_{aw} ($p<0.0001$), only centre contributed a physiologically significant difference. The relationship of sR_{aw} and age, sex and centre was therefore defined as:

$sR_{aw} = 0.455 + 0.044*age + -0.036*sex + -0.41*centre$

(where: centre = coefficient attributed to each centre, age = years, and sex is: 1=male and 2=female).

After adjustment for centre, sex and age were independently associated with sR_{aw}; sR_{aw} decreased with age (β: -0.044 kPa·s, $p<0.0001$), and was slightly lower in females (β: -0.030 kPa·s, $p<0.0001$). Centre explained the most variability (partial $r^2 = 11\%$), compared to 6% for sex and 4% for age. After adjustment for centre, sex and age, sR_{aw} was independent of height (β: 0.002, $p=0.94$). The inter-centre differences contributing to the potentially physiologically significant difference (pre-defined as 0.2 kPa·s) in sR_{aw} outcomes are investigated in the following sections.

5.6.2 Methodological differences

Details regarding equipment and methodology are summarised in Table 5-2.

The Jaeger Masterscreen body plethysmograph (Wurzburg, Germany) was used by all collaborating centres; however a variety of software versions and equipment (masks/mouthpieces/bacterial filters) were used. Due to the retrospective nature of the data collection, the equipment differences could only be investigated in small studies on adults. Software differences and the use of mask/mouthpieces were not thought to contribute to inter-centre differences, whereas neglecting to use a filter appeared to reduce sR_{aw} values (see appendix). Although the investigations of equipment differences were limited, investigations into the impact of applying different methods of data selection and reporting were feasible and are described in detail in the following sections.

Table 5-2: Equipment and methodology used by five collaborating centres.

Centre	Equipment (Jaeger):			Quality control: (selection of tangent)	sR_{aw} outcomes [#]	Reporting of results
	Software Version	Mask or mouthpiece	Use of Filter			
1	V4.1	mouthpiece	Sometimes [##]	computer & manual	sR_{tot} & sR_{eff}	Weighted mean
2	V4.22 & V4.34	Specialised mask	No	Computer	sR_{tot}	Mean of Median
3	V4.34	mouthpiece	No	Manual	sR_{tot}	Median of first set of acceptable loops
4	V4.65 & 5.01	mouthpiece	Yes	Computer	sR_{tot} & sR_{eff}	Weighted Mean, mean of medians & median
5	V4.34 & 4.65	mask	Yes	Computer	sR_{eff}	Mean of Median

[#]Definitions of the difference sR_{aw} outcomes are available in section 5.6.2.1
[##]Filters were used in specific patient groups (e.g. cystic fibrosis), but not in healthy children.

5.6.2.1 Manual or automatic tangent selection

With the exception of 'effective resistance' (sR_{eff}), which is calculated as a regression of pressure and flow over the entire breathing cycle, the sR_{aw} outcome is generally derived from the tangent of the slope of pressure/flow curve (P/F) which can be placed:

- between points of maximum plethysmographic (box) pressure (total resistance, or sR_{tot})
- between peak inspiratory and peak expiratory flow (sR_{peak})
- over some fixed range of flow or over the central linear portion of the breath (most frequently between 0-0.5L.s^{-1} i.e. $sR_{0.5}$)

The most common sR_{aw} outcomes in children are sR_{tot} and sR_{eff}, however with the numerous outcomes available, it is essential to specify which sR_{aw} outcome is being reported, and whether any manipulation of the tangent has occurred, as each outcome represents resistive changes over a different portion of the curve (see section 2.6.5.3 for the different calculations on sR_{aw} outcomes.)

The sR_{aw} tangent of P/F can be selected automatically by the computer software, or can be manually selected/adjusted by the operator as demonstrated in Figure 5-3. Three centres accepted the computer generated tangent between peak to peak box pressure (sR_{tot}). One centre (centre 1) manually adjusted the tangent periodically when the operator felt it was necessary. The final centre (centre 3) applied a very specific protocol for selecting sR_{aw} loops which involved reanalysing the tangent post data collection and modifying each loop to ensure that the tangent was measured over the central linear portion of the curve, generally at flows at or below 0.5L.s^{-1}.

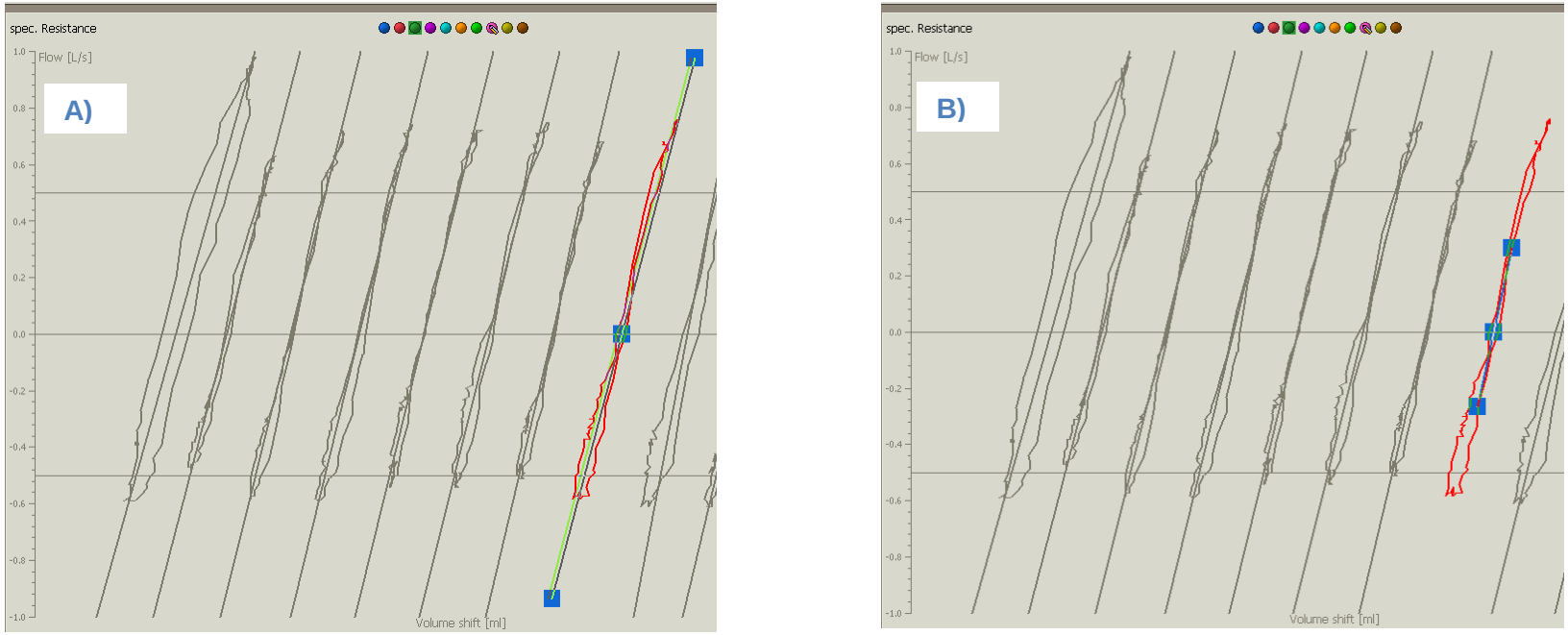

Figure 5-3: Methods for selecting sR_{aw} slope/tangent
A) Computer generated tangent. B) Operator adjusted tangent to represent the central/linear portion of the loop.
sR_{tot} in A was calculated as 0.83kPa·s, whereas sR_{tot} in example B was 0.67 kPa·s

To investigate the effect of manually adjusting the tangent, a subset of 187 randomly selected sR_{aw} measurements were re-analysed by manually adjusting the tangent to measure just the central linear portion of the inspiratory loop (now defined as sR_{mid}). The within-subject comparisons of sR_{tot} (computer generated) and sR_{mid} (manual adjustment) showed sR_{tot} was significantly higher than sR_{mid} (mean difference (95%CI): 0.41 kPa·s (0.38; 0.44)).

A significant correlation ($r^2 = 0.76$, $p<0.0001$) between sR_{tot} and sR_{mid} was observed (Figure 5-4A), and Bland and Altman analysis revealed no bias as sR_{aw} increased. The mean % difference was 38.2% (95% LA: -65.8 ; -10.2%) (Figure 5-4B). Despite the relatively high correlation between sR_{mid} and $sR_{tot,}$ linear regression revealed that sR_{tot} accounted for just 57% of the variation in sR_{mid} (adjusted $r^2 = 0.573$). Marked between-subject variability in this relationship (up to 80% in some cases), together with potential between–operator variability in tangent placement precluded the use of sR_{mid} data, or data which had been manipulated to such an extent. Centres 1 and 3 were therefore excluded from further analysis.

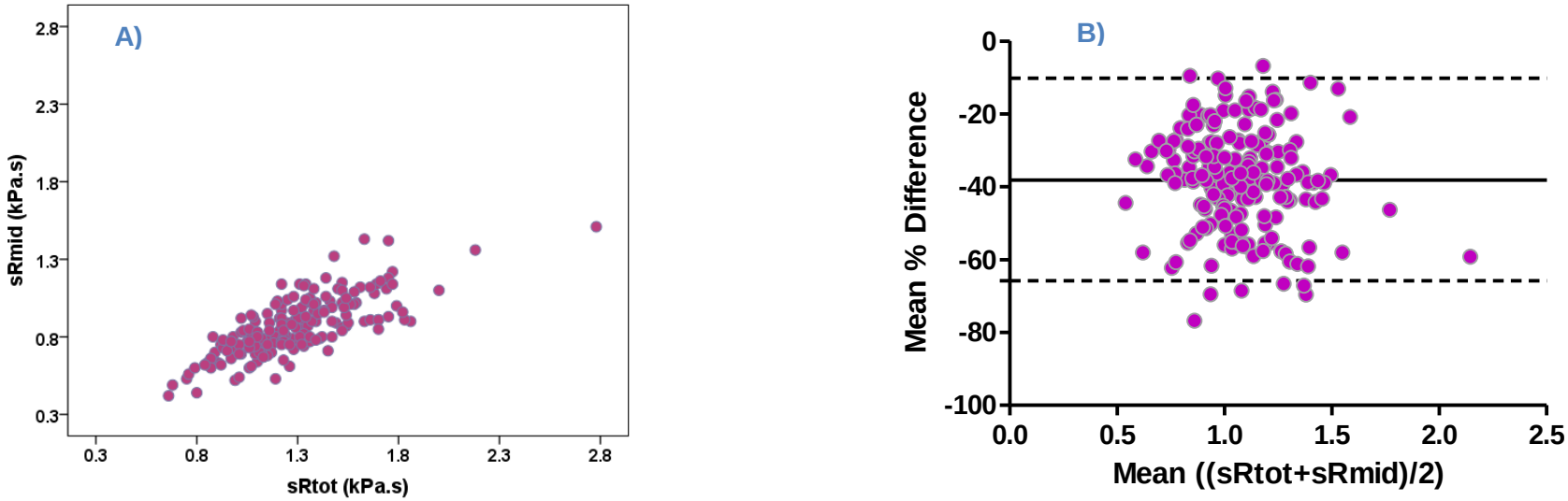

Figure 5-4: Correlation (A) and within-subject comparisons (B) of sR_{tot} (computer generated) and sR_{mid} (manual adjustment).
Legend: (B): Black line indicates the mean difference; dashed lines indicate the 95% limits of agreement.
sR_{tot} and sR_{mid} were significantly correlated (r^2=0.76), however the large within-subject variation (B) precluded the use of sR_{mid} data.

5.6.2.2 Over-read scores

Prior to the final collation of reference data, the three included centres underwent quality control (QC) analysis to ensure data were acceptable. A random sample of 10-20 P/F curves from children studied at each centre were requested to enable a central QC over-read. P/F curves were graded out of 6, with one point given for each of the following criteria achieved:

i. Respiratory rate 30-45 bpm;
ii. Breaths super-imposable (i.e. parallel tangents);
iii. Breaths similar size and shape;
iv. Breaths reasonably closed at zero flow;
v. No obvious distortions (e.g. glottic closure, cough, talking)
vi. Availability of at least two acceptable trials

The over-read sheet and examples of applying the over-read system can be found in Chapter 2, section 2.6.5.4.

Table 5-3 summarises the details of the over-read score from the three centres included in the final dataset. One of these centres did not include breathing frequency on the P/F curve print-outs, but confirmed that the protocol always maintained breathing frequency between 30-45 bpm, so were scored appropriately for this criterion. No data were excluded on the basis of poor over-read scores.

Table 5-3: Summary of QC scores for sR_{aw} loops from the three included centres.

	Centre 2	Centre 4	Centre 5
Number analysed	10	70	18
Resp. Rate = 30-45bpm	8 (80%)	70 (100%)	18 (100%)[a]
Breaths super-imposable	9 (90%)	65 (93%)	18 (100%)
Loops similar size and shape	5 (50%)	60 (86%)	7 (39%)
Loops closed at zero flow	6 (60%)	6 (60%)	0 (0%)
No distortion	7 (70%)	59 (84%)	11 (6%)
>1 set available [b]	10 (100%)	70 (100%)	18 (100%)
Over-all score	5/6	5/6	3/6

Footnote: Results represent the number (%) of subjects from each centre that achieved each QC criteria. [a] Reported to maintain all recordings within 30-45bpm, but not recorded on printouts. [b] i.e. at least 2 technically acceptable trials.

5.6.2.3 Use of different sR_{aw} outcomes: sR_{tot} or sR_{eff}?

Two centres were excluded from analysis due to the protocol they adopted which allowed manual adjustment of the tangent (section 5.6.2.1). Of the three remaining centres, one reported sR_{tot}, one reported sR_{eff} and another reported both sR_{tot} and sR_{eff}. The potential impact of reporting different outcome measures for sR_{aw} was investigated by reanalysing a subset of 228 paired sR_{eff} and sR_{tot} measurements and making within-subject comparisons.

Although a strong correlation ($r^2 = 0.98$) between these two variables was observed, (Figure 5-5A), sR_{tot} was significantly higher than sR_{eff} (mean difference (95%CI): 0.16 (0.15; 0.16) kPa·s)) (Figure 5-5B), hence the two outcomes were not interchangeable. Linear regression of these data revealed the following relationship:

$sR_{tot} = 0.09 + 1.07 * sR_{eff\_}$ kPa·s

The validity of the equation was verified using another dataset which had both sR_{eff} and sR_{tot} measurements. sR_{tot} was calculated from sR_{eff} by applying the regression equation cited above. The newly calculated sR_{tot} ($sR_{tot_}$calc) was highly correlated with the original sR_{tot} values ($r^2 = 0.96$), with no significant difference between them (mean difference (95% CI): 0.007 (-0.01; 0.02)). The validated regression equation enabled adjustment factors to be applied to sR_{eff} and sR_{tot} and allowed a direct comparison of the values (Table 5-4). Values of sR_{eff} and sR_{tot} were similar between the centres, as was the between-subject variability.

Table 5-4: The mean (SD) sR_{aw} values for all included centres.

Centre	sR_{eff} Mean (SD)	sR_{tot} Mean (SD)
2	1.13 (0.3)$_{\#}$	1.29 (0.3)
4	1.09 (0.2)	1.20 (0.3)
5	1.15 (0.2)	1.32 (0.2)$_{\#\#}$

Footnote: $_{\#}$ sR_{eff}: calculated by applying a correction factor to sR_{tot} data: (sR_{eff} = -0.03 +0.9 *sR_{tot})
$_{\#\#}$$sR_{tot}$: calculated by applying a correction factor to sR_{eff} data: (sR_{tot} = 0.09 +1.07*sR_{eff})

In summary, both sR_{tot} and sR_{eff} are useful outcome measures and are highly correlated with one another. To enable future collaboration of sR_{aw} data, future studies should record both outcomes.

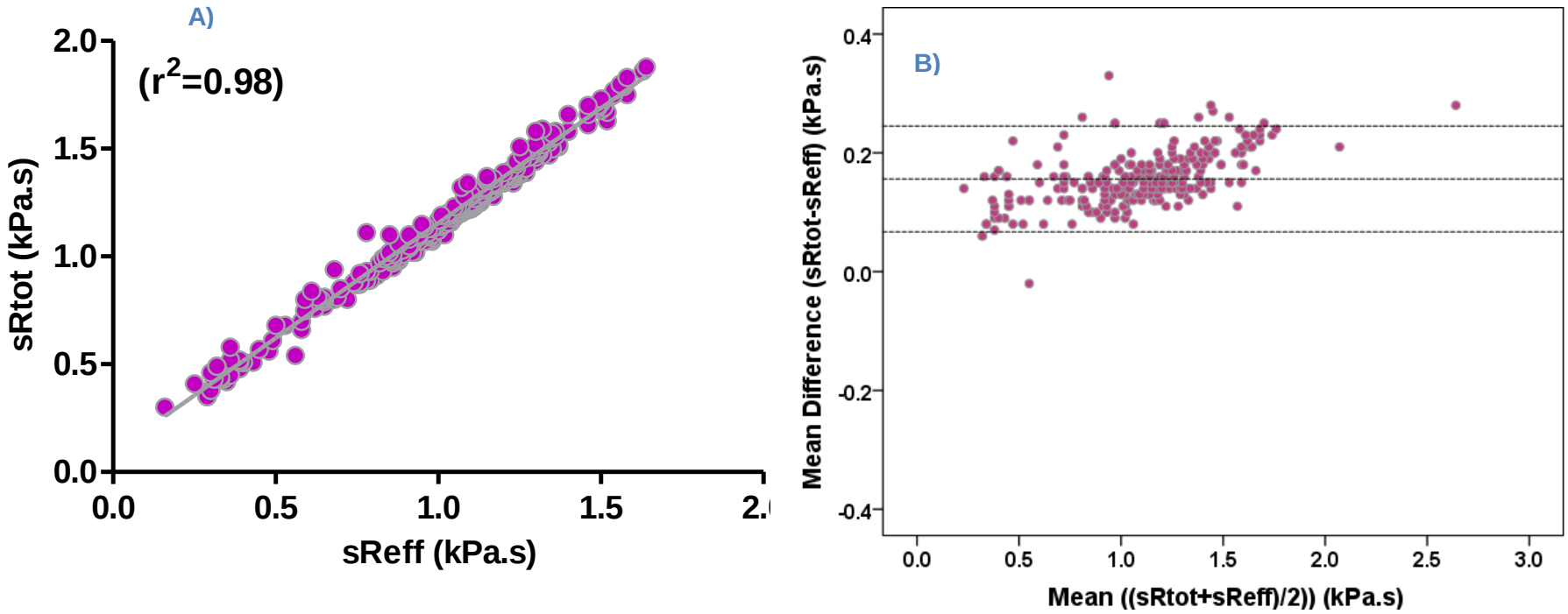

Figure 5-5: Relationship between sR_{tot} and sR_{eff} compared by linear regression (A) and Bland and Altman analysis (B)
Legend: (B): Dashed lines indicate the mean difference and the 95% limits of agreement.
Footnote: sR_{tot} and sR_{eff} were significantly correlated (r^2=0.98), and within subject agreement was low (B). sR_{tot} was significantly higher than sR_{eff}.

5.6.2.4 Different methods of reporting

The collaborating centres demonstrated a variety of methods with regards to reporting results that included:

i. the *weighted mean*, i.e. the sum of all 'acceptable' sR_{aw} values, after rigorous QC, divided by the total number of acceptable values[87]
ii. the *mean of the median* sR_{aw} from three trials, prior to any exclusions
iii. the 'median', as represented by the median value of sR_{aw} from the most representative (i.e. 'median') trial (Figure 5-6)

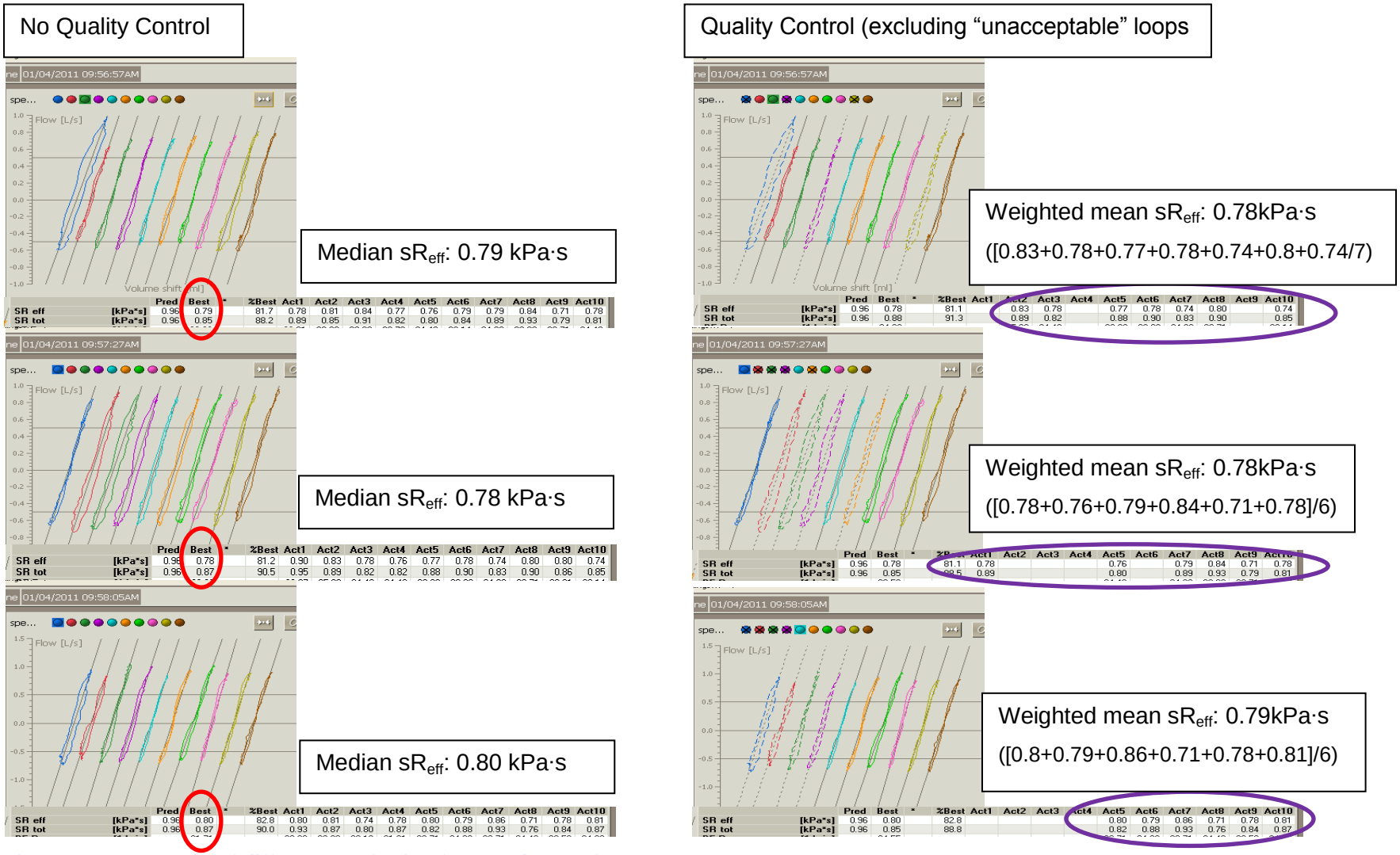

Figure 5-6: Example of different methods of QC and reporting.
Left-hand panel has 3 measurements of 10 sR_{aw} loops. Right-hand panel includes the same measurements but after undergoing rigorous QC.
sR_{eff} with no QC in each measurement was 0.79kPa·s, 0.78kPa·s and 0.80kPa·s (red circles). Median sR_{eff} = 0.79 kPa·s, and the mean-median=0.79kPa·s. Weighted mean is the sum of the acceptable loops (purple circle) divided by the total (n=19) and is calculated as 0.78kPa·s.

The extent to which the method of summarising data influenced reported values was examined by recalculating a subset of 297 sets of results so that both the median and the weighted mean could be compared. Within-subject comparisons revealed no statistical differences between weighted-mean vs. mean-of-median sR_{eff} (mean difference: 0.003 (95%CI -0.001; 0.006) kPa·s) (Figure 5-7). Similar analysis between 101 mean-of-median and median-median sR_{eff} data sets revealed similar results: mean difference: -0.02 (95%CI: -0.07; 0.075) kPa·s .

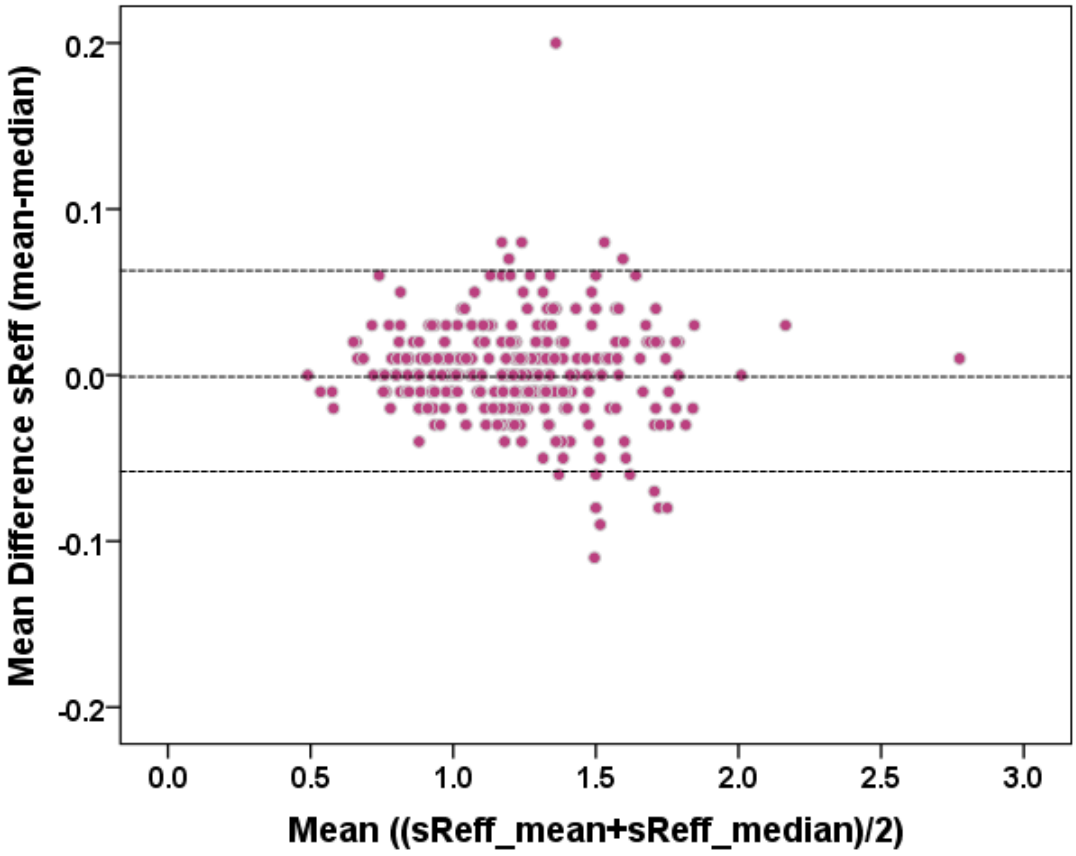

Figure 5-7: Bland and Altman plot comparing methods for reporting results.
Legend: Dotted lines indicate the mean difference +/- 95% limits of agreement.

5.6.3 Summary of the methodological differences

In summary, significant inter-centre methodological differences were observed, which prompted sub-analyses to investigate these differences: The impact of sR_{aw} slope selection was significant across the collaborating centres, with two centres found to adjust the tangent. Despite a significant correlation between sR_{mid} (the value found after adjusting the tangent) and sR_{tot} (computer generated slope) the marked between-subject variability in this relationship, together with potential between–operator variability in tangent placement precluded the use of sR_{mid} data, or data which had been manipulated to such an extent. Subsequently, two centres were excluded from the final collation. The remaining centres underwent specific quality control over-read analysis. Some variation in the QC scores attained between the three centres was observed, however no data were excluded on the basis of poor QC.

The use of different sR_{aw} outcomes and the methods of reporting were then evaluated. sR_{tot} and sR_{eff} were the most common outcomes and were found to correlate well with one another. Although sR_{tot} was significantly higher than sR_{eff}, linear regression enabled the relationship to be defined so that the outcomes could be calculated from one another. In contrast to the significant impact of manually adjusting the slope, the method of reporting results as weighted mean, mean of median or median-median did not impact the final outcome, thus the least time consuming method (median-median) is recommended for future reports, especially as this is more objective and not subject to inter-observer variability.

Data from three centres were collated and reference equations were developed (section 5.7). Further analysis on retrospective data collected at the UCL Institute of Child Health (ICH) to ascertain additional factors which may influence sR_{aw} outcomes were examined prior to generating recommendations in section 5.9.

5.7 Development of reference equations

The Asthma UK initiative collated sR_{aw} data from five international centres. After a thorough investigation of the methodology, two centres (centres 1 & 3) were excluded from the final collation, thus 1908 sR_{aw} measurements from three international centres were available to enable reference equations to be constructed. The equations were constructed by Dr Sanja Stanojevic using the LMS method[133] described in Chapter 2, section 2.9.6. Separate models were developed for males and females, and smoothly changing curves, which explain the age related changes, were developed. The reference equations were limited to children aged 3 to 10 years to avoid edge effects and were created with the prerequisite that measurements were performed under the recommendations described in section 5.9.

Z Scores can be obtained by substituting the values for M, S and L from Table 5-5 into the following equation:

Z Score = [(Measurement/M)L - 1] / [L x S]

Table 5-5: Reference equations for sR_{tot} and sR_{eff} for children aged 3 to 10 years.

	M	S	L
sR_{tot}	1.308 - 0.0001648*age^3 - 0.037*sex	exp(-1.727 -0.00428*age^2)	0.048
sR_{eff}	1.143 - 0.0001369*age^3 - 0.038*sex	exp(-1.651 -.003786*age^2)	0.088

Age is in decimal years; for sex enter 1 for males and 2 for females; exp (exponentiate).

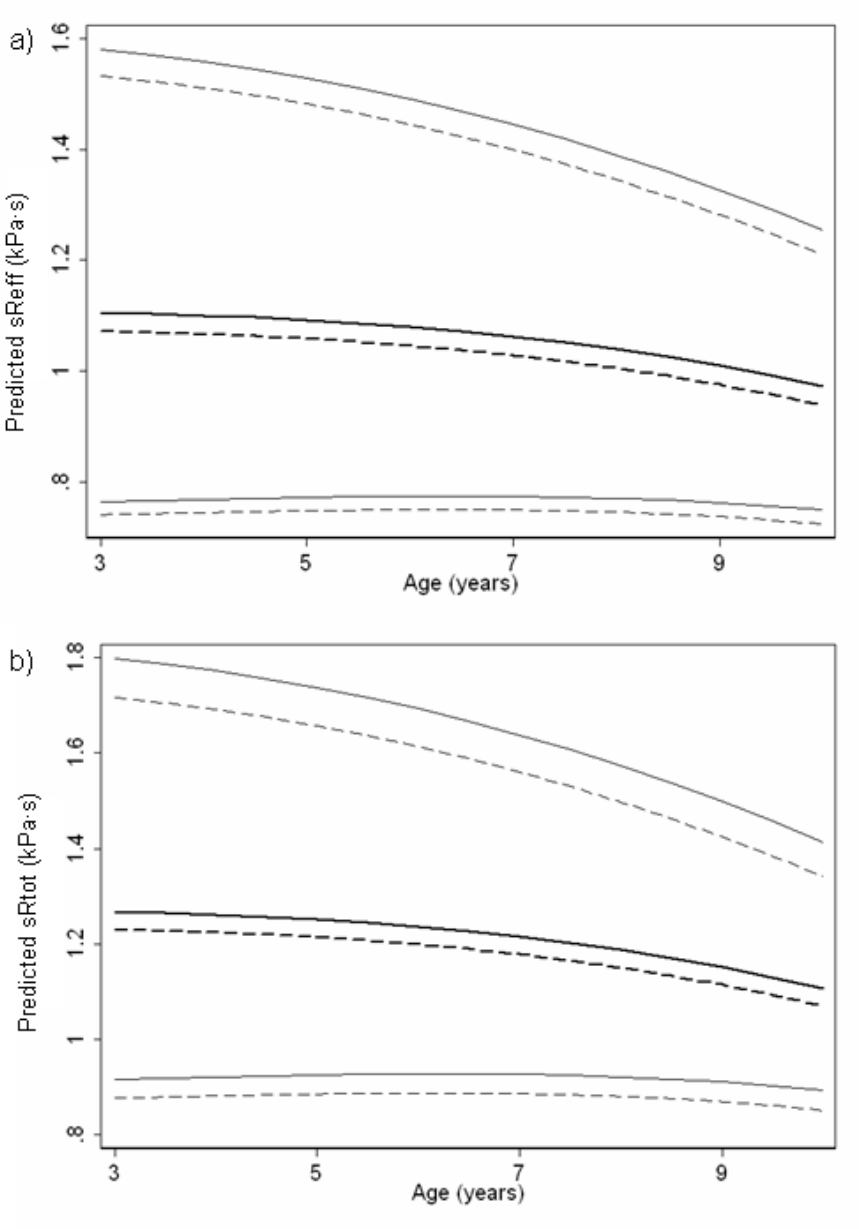

Figure 5-8: Predicted values of sR_{aw} (kPa·s) with upper and lower limits of normal. (a) sR_{eff} and (b) sR_{tot} for children aged 3 to 10 years. Solid lines represent equations for boys, whereas dotted lines represent equations for girls.

5.8 sR_{aw}: Influence of breathing pattern

Previous sections have described the collation of sR_{aw} data in healthy children and the development of reference equations. Prior to developing recommendations to accompany these reference equations, further analysis was conducted to investigate other factors that could potentially influence sR_{aw} outcomes.

Breathing frequency has previously been demonstrated to have a marked impact on measured values of sR_{aw}.[81] The true impact of breathing pattern, however may relate more to flows attained, which can vary markedly while maintaining identical breathing frequency, than to respiratory rate per se. Figure 5-9 demonstrates the impact of breathing pattern in an individual subject. In this case, breathing frequency was doubled but no change in sR_{aw} occurred when the same flows were maintained (Figure 5-9 A&B), however when flows and breathing frequency were increased there was a large increase in sR_{aw} (Figure 5-9 C&D).

Systematic investigation of breathing pattern in the Asthma UK collated data was impeded by the fact that flows were not available as outcome variables within the plethysmographic program, such that readings would need to be taken from print-outs, which were too small to be reviewed. The impact of flows was therefore reviewed retrospectively in children studied at ICH. Additional adult data were used to answer questions difficult to address in children.

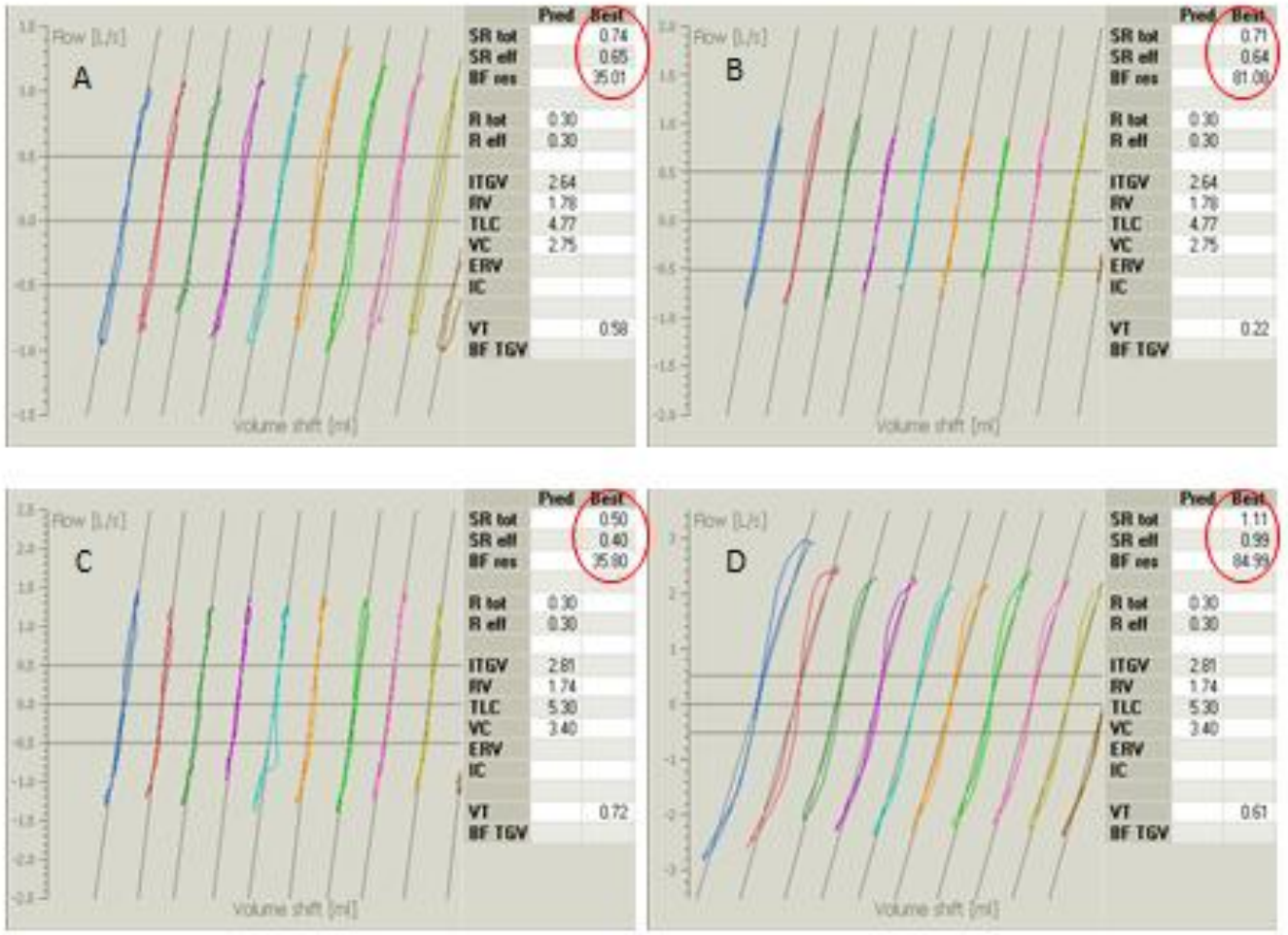

Figure 5-9: Impact of breathing frequency and flows on sR_{aw}.
sR_{aw} remains relatively the same when flows are maintained at -1.0 $L \cdot s^{-1}$ to +1.0$L \cdot s^{-}$ even when breathing frequency is increased from 35bpm (A) to ~80bpm (B). By contrast when flows are approximately doubled, sR_{aw} is also doubled (C and D).

5.8.1 Evaluation of breathing pattern in children

A retrospective analysis of flows obtained during sR_{aw} measurements of 180 White children studied at ICH from five research projects was reviewed. One-way ANOVA analysis revealed significant differences in age across the five projects ($p<0.0005$), however no significant difference was observed in height, weight and BMI when expressed as Z Scores[131] (Table 5-6). As a group, the mean (SD) age, and height, weight and BMI Z Score was 8.3 (2.0) years; 0.2 (0.9); 0.3 (0.8) and 0.3 (0.9) respectively.

Table 5-6: Comparison of demographics, sR_{aw} and breathing pattern.

	1:	2:	3:	4:	5:
Year:	2004-06[129]	2006-07[16]	2006-08[104]	2009-10[144]	2011
n (% male)	23 (57%)	53 (59%)	54 (50%)	40 (48%)	10 (50%)
Age (yrs)	7.5 (0.7)	11.0(0.5)	7.9 (1.2)	6.0 (0.7)***	7.3 (1.8)
Height Z Score	0.4 (1.0)	0.2 (0.9)	0.2 (1.0)	0.0 (0.9)	0.4 (0.8)
Weight Z Score	0.5 (0.8)	0.2 (1.0)	0.2 (0.8)	0.3 (0.7)	0.4 (0.6)
BMI Z Score	0.4 (0.7)	0.2 *1.0)	0.2 (0.8)	0.5 (0.7)	0.3 (1.0)
sR_{tot} Z Score	0.3 (1.0)	0.4 (0.6)	0.5 (0.9)	-0.6 (1.0)***	0.0 (0.7)
sR_{eff} Z Score	0.0 (1.2)	NA	0.5 (0.9)	-0.7 (1.0)***	0.0 (0.7)
BF (bpm)	42.4 (10.8)	33.4(3.7)	43.9(7.8)	37.1 (4.5)***	36 (1.5)
Netflow ($L \cdot s^{-1}$)	1.2 (0.5)	1.2 (0.4)	1.1 (0.4)	0.8 (0.2)***	1.0 (0.3)

Unless stated otherwise, results presented as mean (SD) ***p<0.001
Results based on five research studies conducted at ICH between 2004 and 2011.
BF = Breathing Frequency. Netflow was calculated as ((PIF-PEF)/2) Demographics and sR_{aw} were expressed as Z Scores according to reference equations by the CDC[131] and Kirkby *et al*[128] respectively

Since no quantitative measure of peak expiratory flow (PEF), peak inspiratory flow (PIF) or average flow were reported within the sR_{aw} program, an estimation of PEF and PIF was approximated from the flow axes on scaled printouts and calculating the average flow ("net-flow": (PIF – PEF)/2) for each sR_{aw} measurement (Figure 5-10)

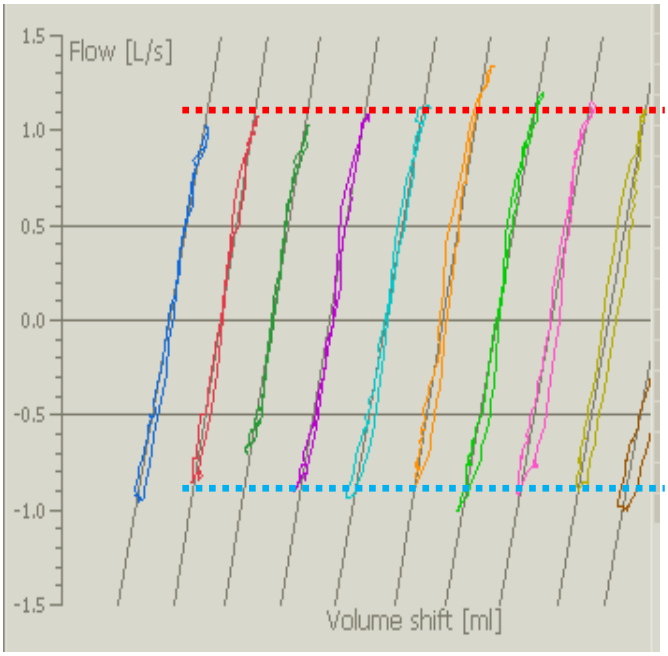

Figure 5-10: sR_{aw} loops from which "net-flow" was calculated.
Legend: Dashed lines identify the estimated peak inspiratory (red) and expiratory (blue) flow, from which "net-flow" was calculated.

All children studied had sR_{tot} measurements within the normal range (+/- 2 Z Scores as defined by section 5.7). However, despite the use of identical protocols and equipment during all projects, significant group differences between projects were observed; these differences being of potential clinical/physiological relevance.

Subsequent investigations into the inter-project differences were undertaken: Project 4 was a preschool project, in which the children were significantly younger, and the flows attained by these children were significantly lower than those children measured in the other projects. After excluding project 4, one-way ANOVA analysis of the remaining four projects revealed no significant group differences in sR_{tot} Z Scores ($p=0.09$) or netflow ($p=0.16$), however significant differences in breathing frequency were still observed ($p<0.0005$). The impact of breathing pattern (breathing frequency and flows attained) and age are investigated in the following sections.

5.8.2 Association of breathing frequency and netflow

Figure 5-9 demonstrated that breathing frequency can vary markedly whilst sR_{aw} values can remain constant as long as flows are maintained relatively constant. As can be seen in Figure 5-11 there was no correlation between netflow and breathing frequency during sR_{aw} measurements ($p=0.73$). The majority of children aged 4 to 11 years adopted a breathing pattern which has a netflow <1.5 $L \cdot s^{-1}$ and breathing frequency <50bpm.

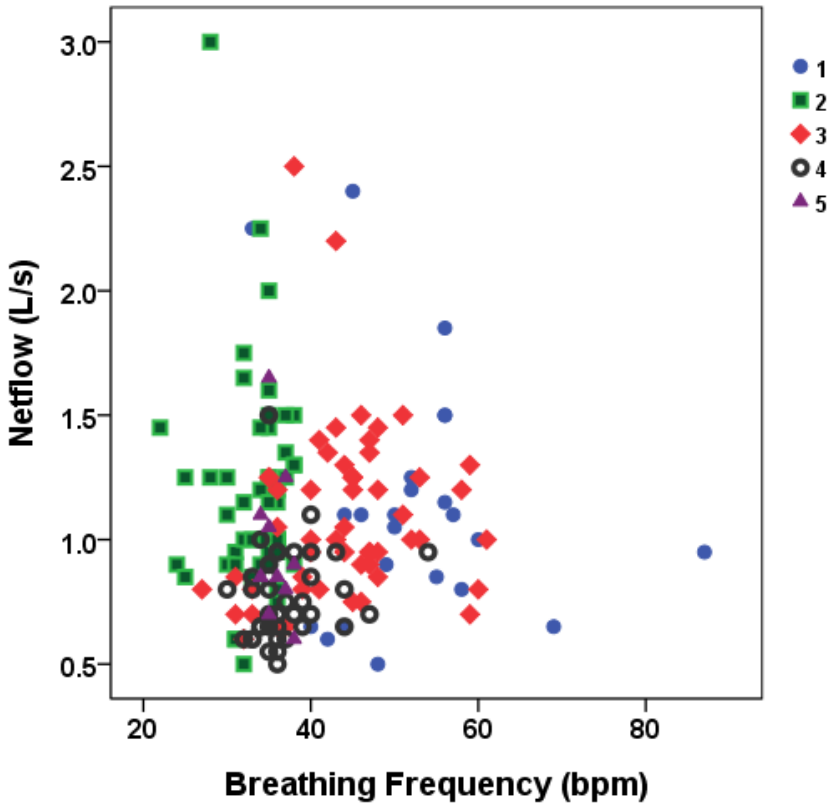

Figure 5-11: Breathing pattern adopted sR_{aw} measurements.
Results based on 180 healthy White children.
No association between netflow ((PIF-PEF)/2)) and breathing frequency was observed (p=0.73)

5.8.3 Adult verification: influence of breathing pattern on sR_{aw}

The impact of breathing pattern on sR_{aw} was investigated in 12 healthy adults (age 17 to 56 years). Repeated sR_{aw} measures at either 30bpm or 60bpm at low flows (i.e. quiet, natural breathing) or high flows (i.e. forced breathing) were performed in a random order. Paired t-tests were used to determine the impact of flow and BF on sR_{eff}.

When flows were doubled from approximately ±1 $L \cdot s^{-1}$ to ~±2 $L \cdot s^{-1}$, while maintaining a constant breathing frequency there was a significant increase in sR_{aw}: mean difference (95%CI): 0.3 kPa·s (0.2; 0.4) p<0.0001) (Figure 5-12A). By contrast, when breathing frequency was doubled while maintaining a constant flow there was no significant change in sR_{aw}: mean difference (95%CI): 0.06 kPa·s (0; 0.1), p=0.21) (Figure 5-12B).

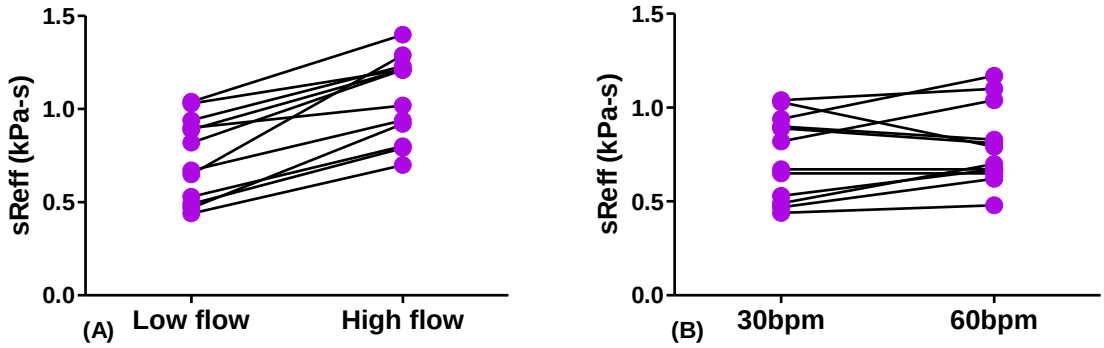

Figure 5-12: Within-subject changes in sR_{eff} when breathing pattern is altered. a)Flows are doubled but breathing frequency remains constant at 30bpm, b) flows remain relatively constant but breathing frequency is doubled.
Doubling flows results in a significant increase in sR_{eff}, whereas doubling the breathing frequency makes little difference to sR_{eff}.

5.8.4 Impact of breathing frequency

Projects which commenced data collection prior to 2006 (projects 1 and 3) were not subjected to the strict quality control and breathing frequency criterion to the same extent as those which started at a later date. Consequently some of these children had adopted a breathing frequency >45bpm (Figure 5-13 A). Despite the variability in breathing frequency across the projects, sR_{tot} Z score remained relatively constant. Although a significant (p=0.018), but weak correlation between breathing frequency and sR_{tot} Z Score was observed (r=0.177), it was unlikely to bear any clinical significance (Figure 5-13 B).

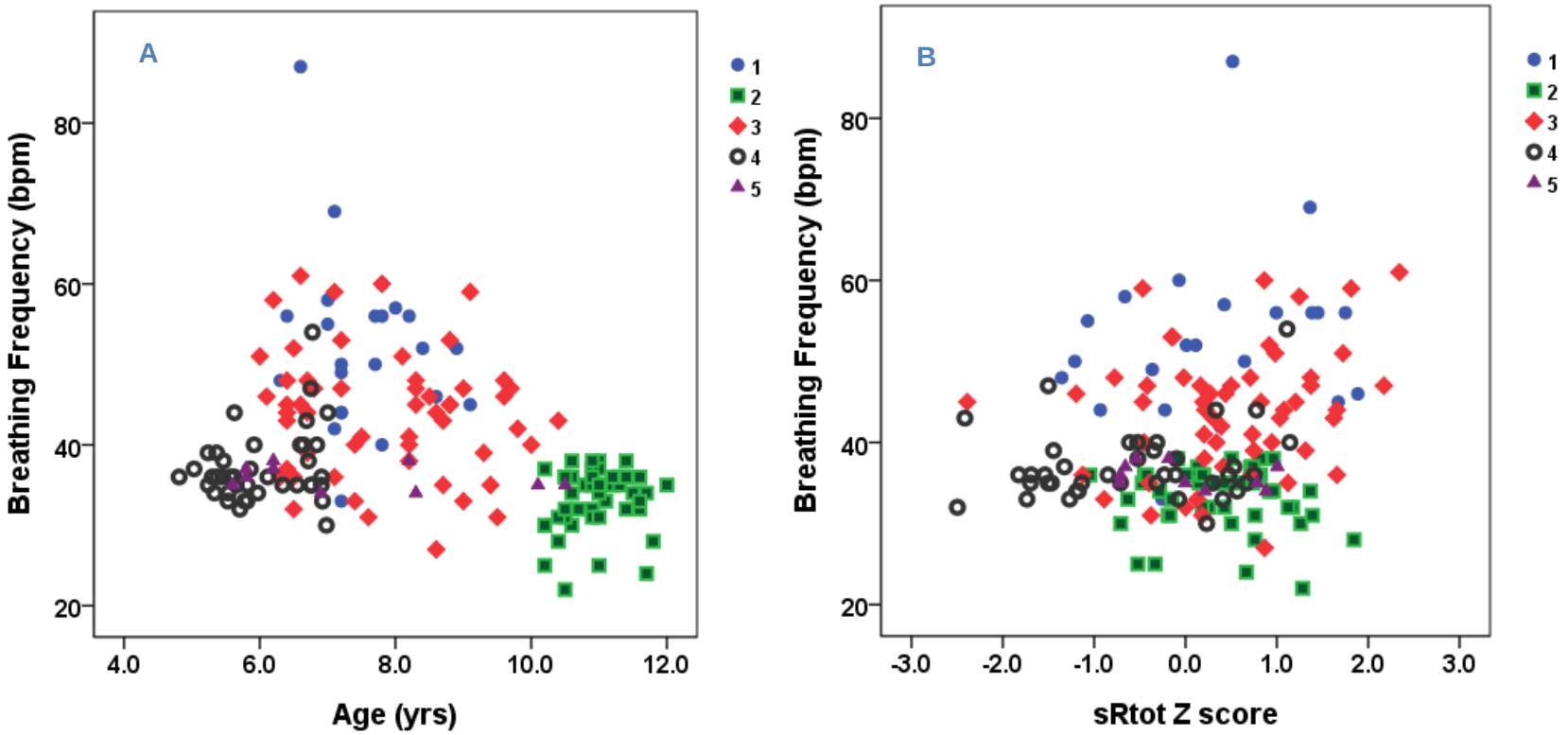

Figure 5-13: Breathing frequency adopted during sR_{aw} measurements in 180 healthy White children according to project.
A) Comparison between breathing frequency and age. B) Comparison between breathing frequency and sR_{tot} Z score.
A significant but weak correlation between sR_{tot} Z Score and breathing frequency was observed.

5.8.5 Impact of netflow

The shallower breathing pattern (i.e. lower netflows) in pre-school children (project 4) compared to the older children is illustrated in Figure 5-13. The pre-school children also had significantly lower sR_{aw} Z Scores. Figure 5-14 plots netflow against age and sR_{tot} Z Score. Pearsons correlation revealed significant ($p<0.0001$), but weak correlations between age and netflow ($r = 0.39$), albeit that the majority of children measured in the age range adopted a netflow of <1.5 $L \cdot s^{-1}$ (Figure 5-14 A). In addition netflow was also weakly correlated with sR_{tot} Z Score ($r = 0.46$, $p <0.0001$) (Figure 5-14 B).

Multiple regression analysis was used to determine how much of the variance in sR_{tot} could be explained by netflow alone, and by netflow, breathing frequency and age in combination. The coefficient for netflow, breathing frequency and age was 0.43, 0.26 and 0.06 kPa·s respectively. Thus only netflow and breathing frequency made a significant, clinically relevant contribution to sR_{aw} ($p<0.0001$).

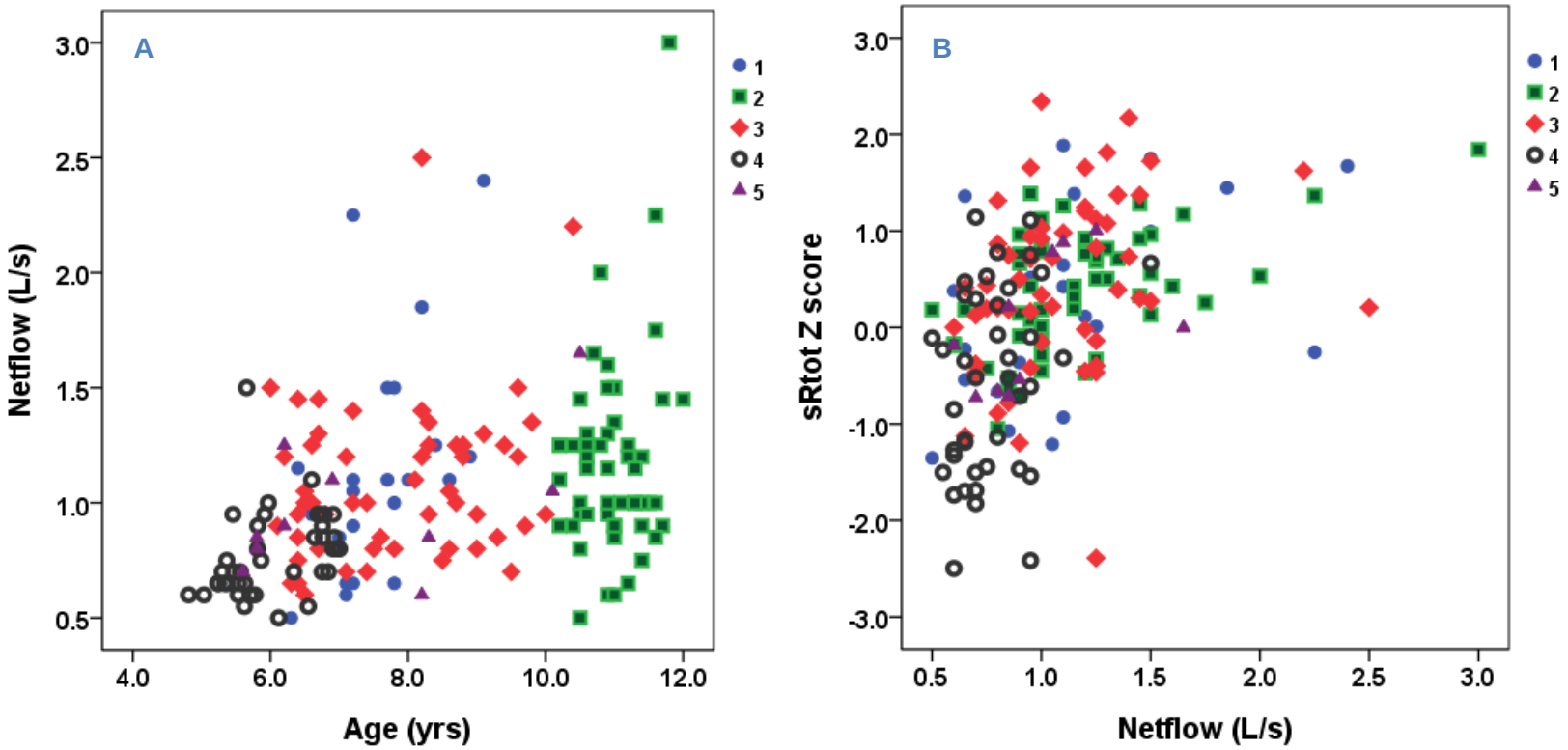

Figure 5-14: Flows attained during sR_{aw} measurements in 180 healthy White children according to project.
A) Comparison between netflow and age. B) Comparison between netflow and sR_{tot} Z score.
The majority of children adopt a netflow of <1.5L·s^{-1}, however there is a weak correlation between age and netflow (A). Higher netflows are also associated with increased sR_{tot} Z Scores.

5.8.6 Summary of the influence of breathing pattern

An investigation into the impact of breathing patterns on sR_{aw} Z Scores was conducted after the Asthma UK collation of sR_{aw} data and reference equations were developed. Retrospective analysis of data collected at ICH plus adult verification studies suggest intra-subject variability of sR_{aw} was more related to flows attained than to breathing frequency *per se*, with higher sR_{aw} values being associated with increased flows.

The majority of children aged 4 to 11 years adopted a breathing pattern which had a netflow <1.5 $L \cdot s^{-1}$ and breathing frequency <50bpm, however results suggest that healthy children may breathe at higher breathing frequencies without increasing sR_{aw} values. Emphasis on breathing pattern may therefore be of more importance than breathing frequency alone. To reduce variability between subjects, and further standardise recordings, subjects should be encouraged to breathe quietly and naturally during sR_{aw} measurement.

5.9 Recommendations for sR_{aw} measurements

Based on the data collated from five international centres, the subsequent exclusion of two centres, and further retrospective analysis of data from different projects within one centre, recommendations for data collection and analysis were developed. These recommendations, together with the reference equations developed by Dr Sanja Stanojevic were published in the *European Respiratory Journal*.[128] The main conclusions were as follows:

i. As software and equipment change, it is recommended that laboratories always validate any major software releases by within-subject comparisons in biological controls. Results of such biological validation should be collated by manufacturers and placed in the public domain. Validation studies should be performed under identical conditions as that in clinical practice

ii. While we were unable to directly compare results obtained with a modified mask and mouthpiece, previous studies have found no difference between these methods.[145] In order to standardise methodology we recommended an appropriately sized mouthpiece and noseclip be used since these are now used routinely for many preschool lung function tests[1] as well as in older children and may be more readily available

iii. Manual adjustment of the tangent for sR_{aw} should be avoided as this is a subjective method of QC and may impact on the results

iv. It is recommended that for future studies, sR_{eff} be the primary outcome measure since this calculates sR_{aw} from multiple points throughout the breathing cycle. However, recording both sR_{eff} and sR_{tot} is advisable for future collaboration of sR_{aw} data

v. Reporting the median breath from the median trial appears to be the most robust approach as it is not influenced by outliers, and avoids the subjective and time-consuming nature of excluding “inadequate” loops

Demographics:

The following test details should be recorded whenever collecting reference data:

- Measured standing height (+sitting height where possible): Recorded to nearest 0.1cm
- Calculated age: Recorded as (date of test) – (date of birth), in years to 1 decimal place.
- Measured weight : Recorded to the nearest 0.1kg
- Sex: recorded and coded consistently
- Gestational age: Recorded as completed weeks
- Birth weight: Recorded to nearest 0.1kg
- Ethnicity: Consensus required for definition

Equipment:

- Make, model and software version needs to be recorded.
    - A biological validation should be performed prior to upgrading software or equipment
- Use an appropriately sized mouthpiece and noseclip. *(a modified mask may also be used if that is current practice, though many centres now report equal success with a mouthpiece)*
- Always use a bacterial filter
    - Ensure calibrations are performed with filter *in situ,* and internal settings have accounted for the filter

Data Collection:

- Ensure the child is sitting upright, with no leak between the lips and mouthpiece
- Cheek supported with child's hands.
    - NB, while not strictly necessary if no occlusion manoeuvres are performed, this is good practice for when plethysmographic assessments include measures of FRC.
- Natural breathing pattern within the specified range of 30-45bpm, avoiding either shallow panting or hyperventilation.
- Collect at least 3 sets of 'technically acceptable' data during with no gross distortion.

Quality Control:

- Use the automatic computer selected tangent.

Use the sR_{aw} over-read sheet in section 2.6.5.4 to “grade” the quality of the measurement

sR_{aw} outcomes:

- Report sR_{eff} as the main outcome measure
  - sR_{tot} should be recorded where available, to facilitate comparisons with published data and provide objective evidence regarding most discriminative outcome in future clinical trials
  - Breathing frequency, peak expiratory and inspiratory flow (PEF and PIF respectively) should be recorded as QC outcomes
- Report the Median of the median: Select the median (middle) breath from the median trial of 3 technically acceptable sets of 5 or 10 breaths

5.10 Applications in healthy Black children

The Asthma UK reference equations developed and presented in section 5.7 were developed for White children aged 3 to 10 years. Ethnic differences were not expected, however the limited data from non-White children at the time precluded an investigation into ethnic differences.

To determine the impact of ethnicity on sR_{aw}, 56 healthy Black children aged 4 to 10 years underwent plethysmographic sR_{aw} measurements in accordance with the recommendations suggested in section 5.9 and results were compared with those from 148 healthy White children previously assessed at ICH. The age range was limited to 4 to 10 years to reflect the age range for which reference equations had been developed. Black children were significantly older, taller, and heavier and had a higher BMI compared to their White peers (Table 5-7).

Table 5-7: Demographics of healthy children undergoing sRaw measurements.

	Black	**White**	**Mean Difference (Black –White) (95%CI)**
n (% male)	56 (64%)	148 (50%)	
Age (years)	8.3 (1.1)	7.3 (1.4)	1.0 (0.05; 1.4)***
Height Z score	0.8 (1.1)	0.2 (1.0)	0.6 (0.4; 0.9)***
Weight Z score	0.9 (1.1)	0.3 (0.8)	0.6 (0.4; 0.9)***
BMI Z score	0.8 (1.0)	0.3 (0.8)	0.5 (0.2; 0.8)***

Unless stated otherwise, results presented as mean (SD) ***p<0.001
Demographics expressed as Z Scores according to the CDC anthropometric reference equations[131]

Despite the demographic differences, and a significant difference in breathing frequency, there were no significant differences in sR_{eff} measurements obtained from Black and White children (Table 5-8 and Figure 5-15).

Table 5-8: Comparison of sR_{aw} results in healthy children aged 4 to 11 years.

	Black	White	Mean Difference (Black –White) (95%CI)
sR_{eff} (kPa·s)	1.1 (0.2)	1.2 (0.3)	-0.1 (-0.2; 0.0)*
sR_{eff} Z Score[128]	-0.1 (0.8)	0.1 (1.0)	-0.2 (-0.5; 0.1)
Netflow ($L.s^{-1}$)	0.9 (0.3)	0.9 (0.4)	0.0 (-0.2; 0.1)
Breathing frequency (bpm)	35.4 (3.8)	42.2 (9.3)	-6.7 (-0.3; -4.2)***

Unless stated otherwise, results presented as mean (SD) *p<0.05, ***p<0.001
Results based on 56 healthy Black children and 148 healthy White children aged 4 to 11 years.

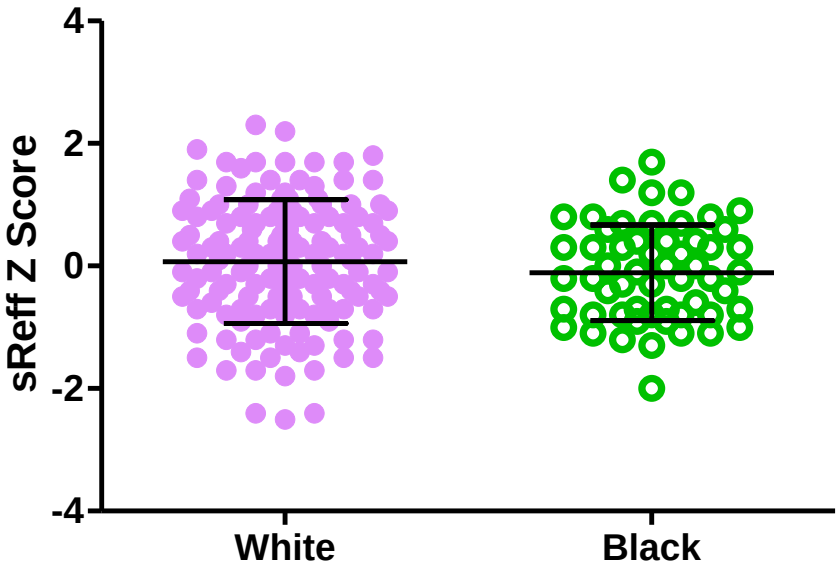

Figure 5-15: Comparison of sR_{eff} Z Scores in healthy White and Black children.
Legend: Black lines denote mean +/-SD.
No significant difference was observed (p=0.22)

In summary, the sR_{aw} reference equations based on healthy White children appear to be applicable for use in healthy Black children of the same age.

5.11 Applications of sR_{aw} in SCD

The previous sections described the development of sR_{aw} reference data for White children, and demonstrated the applicability of these reference equations in healthy Black children. The following section will describe the use of sR_{aw} measurements in children with SCD. All measurements were undertaken under the conditions described in section 5.9 and the reference equations described in section 5.7 were used.

Ninety-nine children with SCD less than 10 years of age were compared with 56 healthy Black children (previously evaluated in section 5.10). The children with SCD were significantly shorter, lighter and had lower BMI than healthy children of the same ethnicity (Table 5-9).

Table 5-9: Comparison of demographics of 56 healthy Black children and 99 children with SCD aged 4 to 10 years in whom sR_{aw} measurements were obtained.

	Healthy Black	SCD	Mean Difference (SCD-health) (95%CI)
n (% male)	56 (64%)	99 (45%)	
Age (years)	8.3 (1.1)	7.6 (1.8)	-0.7 (-1.2; -0.2)**
Height Z score	0.8 (1.1)	0.1 (1.0)	-0.7 (-1.0; -0.3)***
Weight Z score	0.9 (1.1)	0.1 (1.0)	-0.9 (-1.2; -0.6)***
BMI Z score	0.8 (1.0)	0.0 (1.0)	-0.7 (-1.1; -0.4)***

Unless stated otherwise, results presented as mean (SD) **p<0.01, ***p<0.001
Demographics expressed as Z Scores according to the CDC anthropometric reference equations[131]

Other than a slight trend towards children with SCD adopting a gentler breathing pattern (netflow difference: $-0.1 L \cdot s^{-1}$ (p=0.06)), there were no significant differences in sR_{aw} outcomes (Table 5-10 and Figure 5-16).

Table 5-10: Comparison of sR_{aw} measurements in 56 healthy Black children and 99 children with SCD aged 4 to 10 years.

	Healthy Black	SCD	Mean Difference (SCD-health) (95%CI)
sR_{tot} (kPa·s)	1.1 (0.2)	1.1 (0.3)	0.0 (-0.1; 0.1)
sR_{tot} Z Score	-0.1 (0.8)	-0.3 (1.0)	-0.2 (-0.5; 0.1)
sR_{eff} (kPa·s)	1.0 (0.2)	1.0 (0.3)	0.0 (-0.1; 0.1)
sR_{eff} Z Score	-0.1 (0.9)	-0.3 (1.1)	-0.3 (-0.6; 0.1)
Netflow ($L.s^{-1}$)	-0.9 (0.2)	-0.8 (0.2)	0.1 (0; 0.2)
BF (bpm)	35.4 (3.8)	37.6 (7.9)	2.1 (-0.1; 4.3)

Unless stated otherwise, results presented as mean (SD)

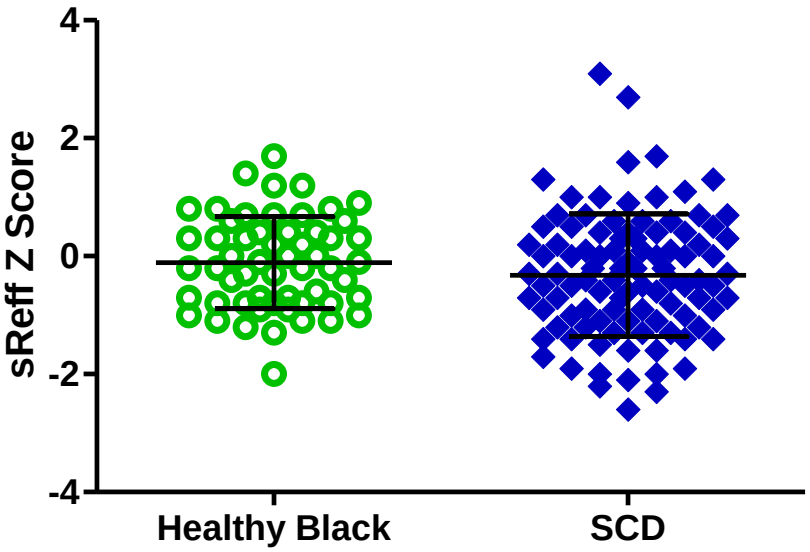

Figure 5-16: Comparison of sR_{eff} Z Scores in healthy Black children SCD.
Results based on 56 healthy Black children and 99 children with SCD aged 4 to 10 years. There was no significant difference in sR_{eff} Z Scores[128] between healthy Black children and children with SCD. The two outliers in the SCD group (with elevated sR_{eff} had concurrent reduction in FEV_1)

These results suggest that baseline sR_{aw} values are unaffected by SCD with just two children with SCD (1%) demonstrating elevated $sR_{eff.}$ Baseline sR_{aw} assessments may not be a useful outcome measure to assess group differences in lung function between healthy children and children with SCD.

5.12 Repeatability and the bronchodilator response

The chapter so far has described the development of recommendations and reference data for sR_{aw}, and demonstrated no differences in sR_{aw} outcomes between healthy Black and White children, and no differences between healthy Black children and those with SCD. A further potential application of sR_{aw} measurements would be as an outcome measure for the assessment of bronchodilator response (BDR). Establishing a clinically relevant BDR requires knowledge of the between-test, within-occasion repeatability in health and disease, and the extent to which (if any) a BDR occurs in healthy children. The following section first reviews the within-test, within occasion repeatability, and then reviews a subset of healthy children and children with SCD who underwent repeated sR_{aw} measurements at ICH with and without a bronchodilator.

5.12.1 Within-test repeatability

The sR_{aw} protocol includes reporting the median of three baseline measurements. The within-test, within-subject repeatability of these three repeated measures at baseline was defined in terms of absolute differences (maximum sR_{eff} minus the minimum sR_{eff}) in kPa·s; percent difference (([highest-lowest]/highest)*100); and the coefficient of variation (CV) (Table 5-11). Due to the uneven distribution of the data, non-parametric tests were performed: Mann Whitney comparisons between healthy Black and White children revealed no significant differences in any of the outcomes (p=0.2; 0.7; and 0.5 for absolute difference, % difference, and CV respectively). In addition, a comparison between healthy children and children with SCD revealed no significant differences in within-test repeatability when defined as absolute difference (p= 0.24), % difference (p= 0.9), or CV (p= 0.7). No significant correlation between age and within-test repeatability was observed in this age group (Figure 5-17 and Figure 5-18). Similarly increased within-test repeatability was not associated with increased sR_{eff} Z Scores (Figure 5-18B).

Table 5-11: Within-test repeatability of sR_{eff} measurements in health and SCD.

	Absolute difference (kPa·s)	Absolute Percent difference (%)	Coefficient of variation (%)
Healthy Black	0.14 (0.08; 0.18)	11.9 (6.6; 15.0)	6.4 (3.6; 8.6)
Healthy White	0.16 (0.10; 0.22)	11.8 (8.0; 17.6)	6.8 (4.5; 10.6)
SCD	0.13 (0.09; 0.21)	11.7 (9.9; 16.4)	6.8 (4.8; 9.8)

Results presented as median (inter-quartile range)

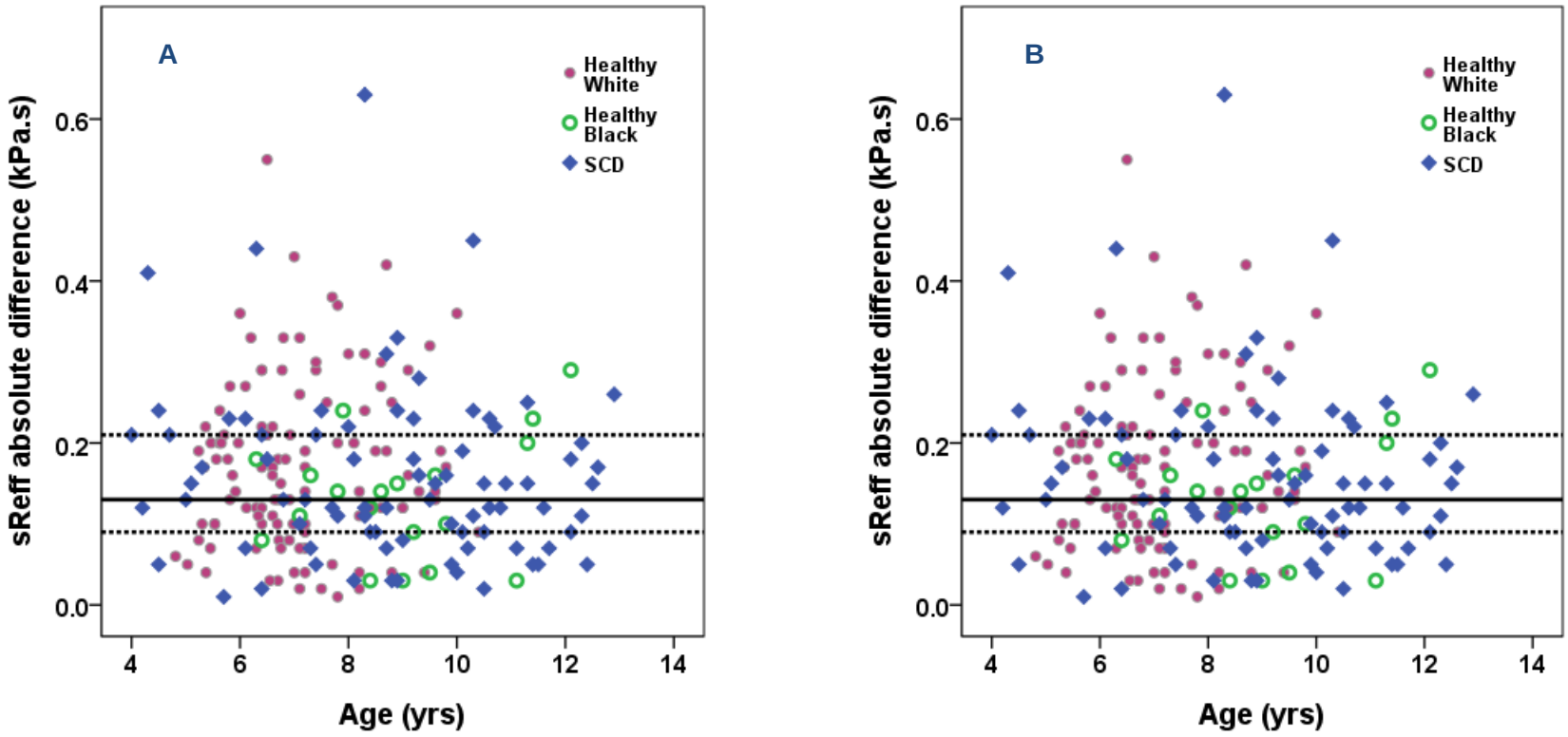

Figure 5-17: Within-test repeatability expressed as A) absolute difference and B) % difference in healthy children and children with SCD.
Legend: Solid Black line indicates the median, dashed lines indicate the inter-quartile range.
No significant correlation between age and absolute difference (p=0.4; r=-0.06) and age and % difference (p=0.5; r=-0.05) was observed

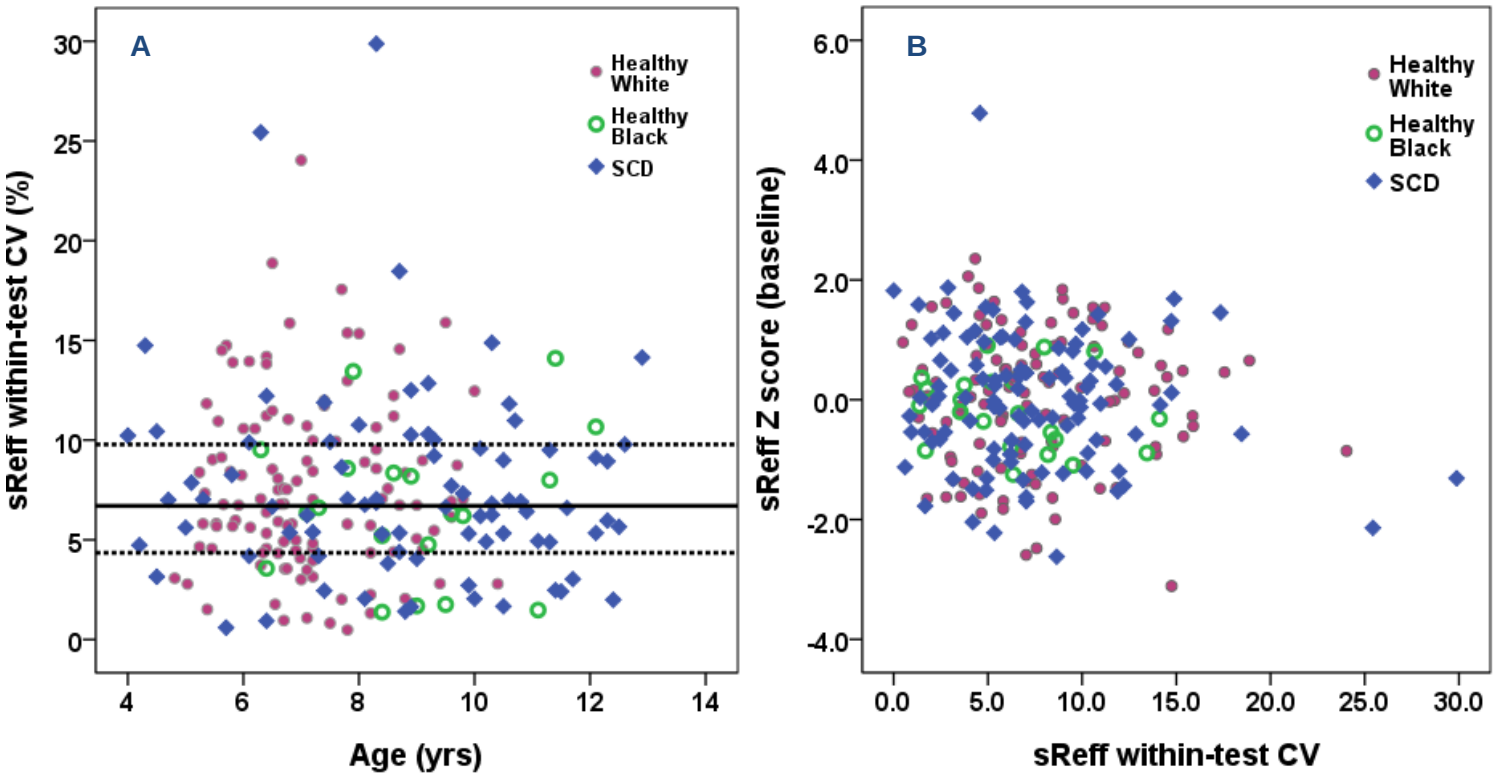

Figure 5-18: A) sR_{aw} within-test repeatability expressed as the coefficient of variation (CV). B) Association of CV and sR_{eff} Z Scores.
Legend: Solid Black line indicates the median, dashed lines indicate the inter-quartile range.
No significant correlation between age and within test CV (p=0.4; r=-0.05) was observed. Similarly an increased CV was not associated with increase sR_{eff} Z Scores. There was no difference according to ethnic group or clinical status.

5.12.2 Between-test repeatability and BDR in health

Between-test sR_{aw} repeatability (i.e. repeated measures 15 minutes after baseline without any intervention) was measured in 20 healthy Black children with a mean age (SD) of 8.7 (1.3) years. Mean (SD) sR_{eff} was 1.28 (0.20) kPa·s at baseline and 1.20 (0.20) kPa·s when repeated 15 minutes later (without any intervention). There was no significant difference between the two repeated measurements (mean (SD) difference (repeat-baseline) was -0.08 kPa·s (0.15) or -2.0% (3.4)) (Table 5-12). Bland and Altman analyses were used to calculate the 95% limits of agreement for the between test repeatability (Figure 5-19A).

Twenty-three healthy children (mean age: 8.3 (1.3) years) underwent BDR assessments with sR_{eff} as the main outcome. Paired t-tests revealed a statistically significant mean difference post bronchodilator (mean (SD) difference: -0.32 kPa·s (0.19)). The 95% limits of agreement established from the between-test repeatability (Table 5-12) were used to identify those children in whom a clinically significant BDR was observed. Eight (35%) of the healthy children demonstrated a clinically significant BDR (i.e. results fell below the 95% limits of agreement established from the between-test repeatability), and all children demonstrated a trend for lower sR_{eff} results post bronchodilator (Figure 5-19B).

Table 5-12: Between test repeatability and BDR of sR_{aw} in healthy children.

	Mean Diff(SD) from baseline (kPa.s)	**95% Limits of Agreement**	
		Lower limit (95%CI)	**Upper limit (95% CI)**
Between-test repeat sR_{eff} (kPa·s)	-0.08 (0.15)	-0.36 (**-0.43**; -0.30)	0.21 (0.14; **0.27**)
Post bronchodilator sR_{eff} (kPa·s)	-0.32 (0.19)***	-0.69 (**-0.77**; -0.61)	0.05 (-0.03; **0.13**)

***p<0.001

Results based on 20 healthy children (between test) and 23 healthy children (BDR) aged 4 to 12 years. 95% confidence intervals around the lower and upper limit of normal were calculated from the standard error of the mean (SEM) and provide a more precise estimation of the limits of normality. Values in bold indicate the most conservative limits, that take the actual sample size of this study into account.

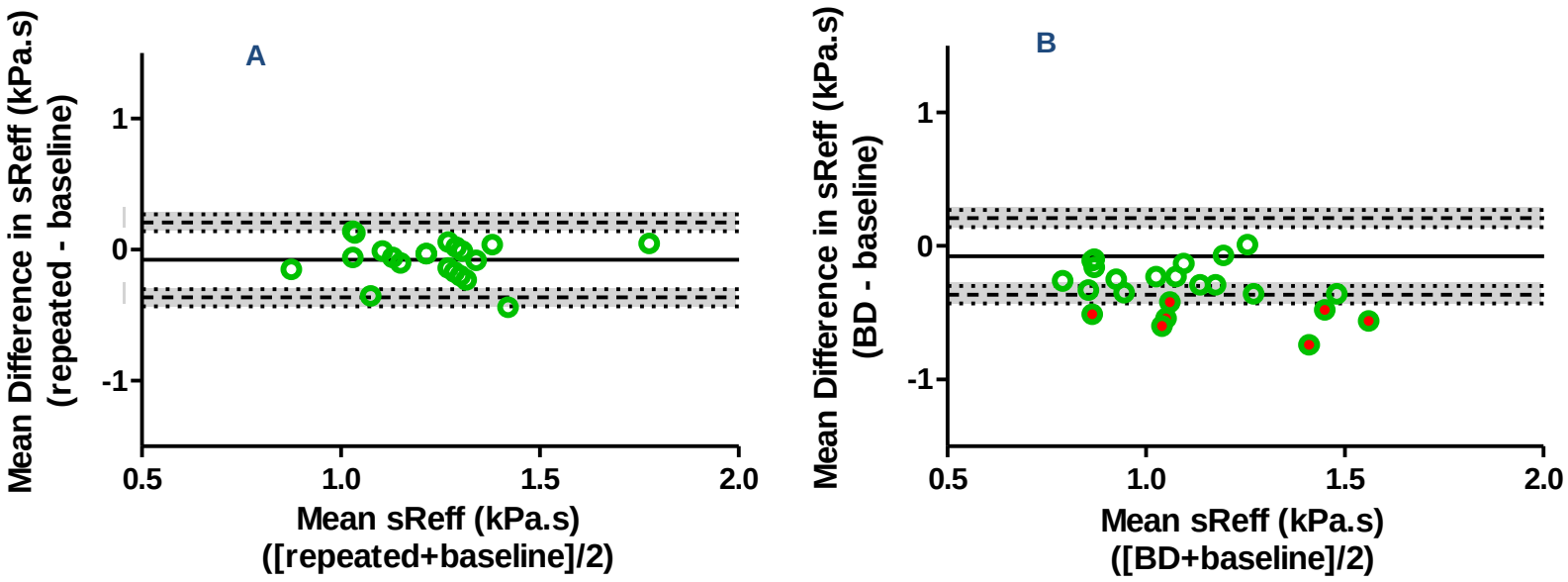

Figure 5-19: A) sR_{aw} between-test repeatability and B) BDR in healthy children
Legend: Dotted lines indicate the mean +/- 95% limits of agreement, grey shaded area indicate 95% CI around the upper and lower limits of agreement based on the between-test repeatability in health. Values that fall below these lines in B (red symbol) denote a significant BDR. The majority of healthy children demonstrated a trend towards a decrease in sR_{eff} post BD. Eight (35%) healthy children demonstrated a significant bronchodilator response (results below the 95% LA established by between-test repeatability).

5.12.3 Bronchodilator response in SCD

A subset of 37 children (49% male) with SCD underwent BDR assessments using sR_{eff} as an outcome measure. Although slightly older than the entire population, mean (SD) age was 9.1 years (1.1), demographics were similar to the main group (Table 5-9). The mean (SD) sR_{eff} was 1.1 (0.3) kPa·s at baseline and 0.9 (0.20) kPa·s post BD. Paired t-tests revealed a statistically significant mean difference (95% CI) of -0.21 kPa·s (-0.30; -0.04) ($p<0.0001$). If expressed as percent change the mean (SD) change post bronchodilator in children with SCD was -16.3 (20.7)%, whereas in healthy the mean (SD) BDR was 19.8 (23.4)%

Although a statistically significant BDR in children with SCD was observed, the clinical relevance of this could only be interpreted when comparing it to the BDR observed in healthy children of similar age and ethnicity. A clinically significant BDR is a response over and above that seen in health, and was estimated from the 95% limits of agreement determined by Bland-Altman analysis on baseline and post BD results in healthy children (Table 5-12). A conservative threshold for the significant BDR (including the 95% CI, which adjusted for sample size), was therefore estimated as -0.77 kPa·s (Figure 5-20A). Only one child with SCD (and concurrent asthma symptoms) fell below the BDR threshold, whilst the remaining children demonstrated little bronchodilator responsiveness (Figure 5-20B).

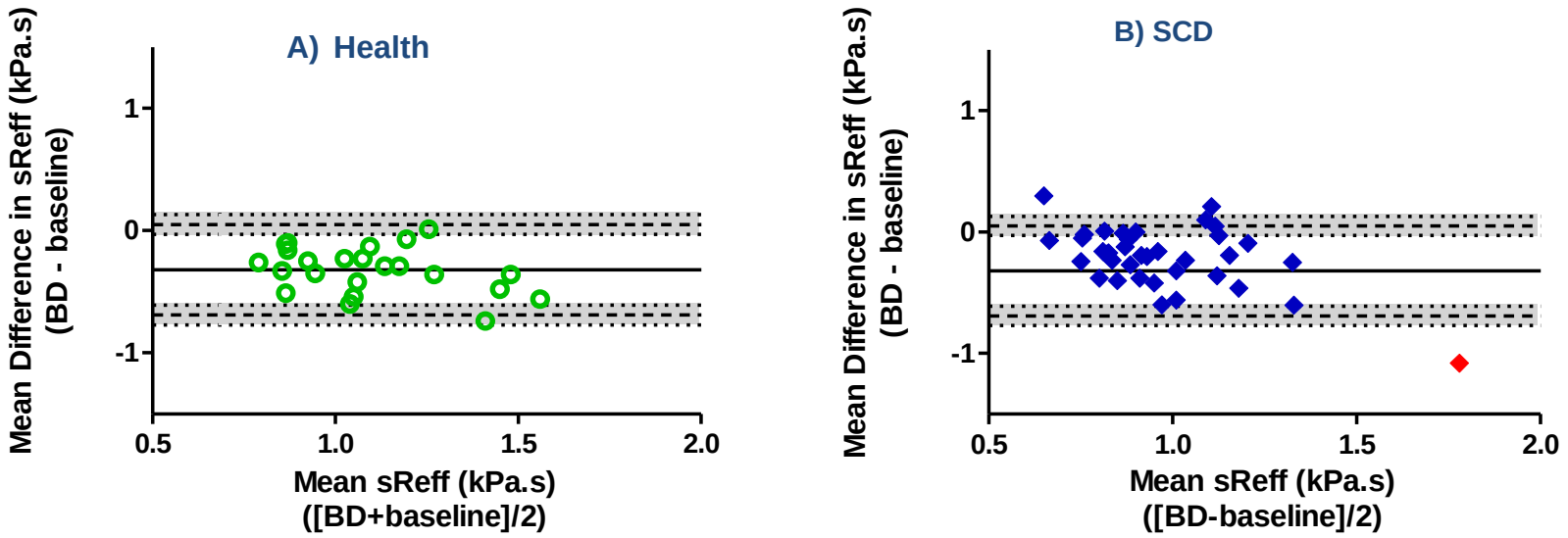

Figure 5-20: A) Between-test sR_{aw} repeatability and corresponding 95% limits of agreement. B) BDR in SCD.
Legend: The threshold for a clinically significant bronchodilator response is defined by the limits of agreement in A.
A) The dashed lines indicate the 95% limits of agreement, and the grey shaded area is the 95%CI around them.
B) Red symbol identifies the child in whom a significant bronchodilator response was observed (i.e. results fell below the lower 95% LA established in healthy children).

5.12.4 Repeatability and BDR: Summary

Within-test repeatability was described in healthy White and Black children as well as children with SCD and was defined in terms of absolute and percentage differences (frequently used in the clinical scenario when the technician requires a quick calculation to determine if results are within acceptable repeatability criteria) and coefficient of variation (CV), which is more commonly used in epidemiological studies to describe the repeatability of a group of subjects. No ethnic differences or differences between health and disease were observed. Based on these results repeated baseline measures of sR_{aw} should be within 0.2 kPa·s or 16% of the highest value (this equates to a CV of 9%).

Between-test repeatability was described in twenty healthy children as ~0.1 kPa·s (2%). The 95% limits of agreement were used to ascertain bronchodilator responsiveness in health, and subsequently 35% of the healthy children assessed were found to have a physiologically significant BDR. The 95% limits of agreement were therefore calculated based on the baseline and post BD sR_{eff} results and used to define the thresholds for reversibility in SCD. Only one child with SCD demonstrated a significant BDR, with the rest demonstrating bronchodilator responsiveness similar to that seen in health. These results are investigated further, in combination with the other lung function tests and the clinical status in Chapter 8.

5.13 Summary

The Asthma UK initiative comprised the largest collation of paediatric sR_{aw} data from healthy controls to date. A comprehensive review of the different methodologies across the five collaborating centres was undertaken which revealed significant between centre differences and necessitated exclusion of data from two centres. Results from the study enabled the development of a quality-control over-read sheet and recommendations for future measurements. Furthermore, preliminary sex-specific reference equations which adjusted for the minimal age-related changes in sR_{eff} and sR_{tot} were developed. This study demonstrated that collation of healthy data and subsequent development of reference equations is possible, but highlighted the potential for increased inter-centre variability unless data collection/conditions are standardised.

In addition to developing recommendations and reference equations, sR_{aw} measurements were applied in healthy Black children and children with SCD. No differences in sR_{aw} between healthy White and Black children were observed, thus enabling the reference equations to be applied and interpreted with some confidence in Black children with suspected lung disease. sR_{aw} measurements did not, however distinguish children with SCD from healthy controls, nor was it shown to be a useful outcome measure in bronchodilator responsiveness assessments in this group of SCD patients.

This study demonstrates that sR_{aw} measurements in Black children with suspected lung disease are feasible and can be interpreted using published reference data. However, sR_{aw} measurements do not appear to be a useful outcome measure in the clinical management of SCD. The combination of sR_{aw} measurements with other lung function tests are investigated further in Chapter 8.

6 Plethysmographic Lung Volumes

6.1 Introduction

Plethysmographic lung volume measurements are the gold standard for identifying restrictive lung disease.[94] Interpretation of these measurements in non-White children however, may be limited due to the fact that the published reference data, based on White children only,[97,98] have been shown to be unreliable.[16,99,104] In addition, to our knowledge, there are no plethysmographic reference data available for Black children. Furthermore, while guidelines for quality control (QC) and repeatability have previously been defined in adults,[94] the appropriateness of these QC criteria in children have not been formally assessed.

This chapter will evaluate the available paediatric reference data in healthy Black and White children, and assess the impact of ethnicity on lung volumes. These data have been published in *Pediatric Pulmonology*.[146] The use of plethysmographic lung volumes in children with regards to applying the adult QC criteria and repeatability measures[94] will then be reviewed, prior to the development of recommendations for applying these measurements in children with suspected lung disease. These recommendations are then applied in children with Sickle Cell Disease (SCD) to evaluate the clinical role of plethysmographic lung volume measurements in these children.

6.2 Aims

The primary aims were:

i. To evaluate published paediatric plethysmographic lung volume reference data[97,98]
ii. To investigate potential ethnic differences in plethysmographic lung volumes and develop recommendations for interpreting plethysmographic lung volumes in Black children
iii. To evaluate the extent to which plethysmographic lung volume measurements detect lung disease in children with SCD

The secondary aims were:

i. To determine within-test repeatability in healthy children and children with lung disease
ii. To develop and apply a QC over-read scoring system and assess the feasibility of current guidelines[2]

6.3 Objectives

The primary objective was to compare lung volume data in healthy Black children to published reference data based on White children.[97,98] To achieve this objective, data were collected in two international centres (London and St Louis, USA) and inter-centre comparisons were undertaken to determine if data could be combined. Secondary objectives were to over-read lung volume data in healthy children and children with SCD to ascertain the appropriateness of applying adult QC and repeatability criteria in children and develop recommendations for future plethysmographic lung volume measurements.

6.4 Hypothesis

Lung volume data from healthy Black children will be significantly lower than that predicted by reference data derived from White children.

6.5 Subjects and sample size

Plethysmographic lung volumes were collected in healthy Black children aged 6 to 12 years from two international centres (The UCL Institute of Child Health, London, UK and Washington University, St Louis, USA) as part of the SAC study and SLIC study (described previously in chapter 2, section 2.3). In addition, data collected from healthy White children of the same age from previous on-going research projects were collated.[16,104,129]

A sample size of 64 children in each group (Black and White children) were required to determine differences in outcomes equivalent to 0.5SD (which equates to ~5% predicted in FRC and TLC) with 90% power at the 5% significance level.

6.6 Collation of lung volume data: Inter-centre comparisons

Plethysmography was performed in 68 healthy Black children (40 from ICH and 28 from the USA). The plethysmographic lung volume outcomes reviewed were:

- Functional Residual Capacity (FRC) (L)
- Residual Volume (RV) (L)
- Total Lung Capacity (TLC) (L)
- Ratio of RV to TLC (RV/TLC)

Unpaired t-tests revealed that the Black children measured in the USA were significantly older than those measured in the UK, however, when results were adjusted for age and sex, there were no significant differences in height, weight and BMI Z Scores[131] (Table 6-1).

Table 6-1: Demographics of 68 healthy Black children in whom plethysmographic lung volumes were obtained, according to measurement site.

	UK Lab	USA Lab	Mean Diff (UK - USA) (95%CI)
n (% male)	40 (40%)	28 (46%)	
Age (years)	9.4 (1.4)	10.7 (1.2)	-1.3 (-1.9; -0.6)***
Height Z Score	0.8 (1.1)	0.4 (1.1)	0.4 (-0.2; 0.9)
Weight Z Score	0.8 (1.1)	0.8 (1.0)	0.0 (-0.5; 0.5)
BMI Z Score	0.6 (1.1)	0.8 (0.9)	-0.2 (-0.7; 0.3)

Unless stated otherwise, results presented as mean (SD) ***p<0.001
Demographics were expressed as Z Scores according to the CDC reference equations[131]

There were no significant differences in plethysmographic lung volumes between the two centres in absolute values or when corrected for sex and height (based on Rosenthal reference equations[98]) (Table 6-2).

Table 6-2: Comparison of Plethysmographic lung volume data in healthy Black children from the UK and the USA.

	UK Lab	USA Lab	Mean Diff (UK-USA) 95% CI
n (% male)	40 (40%)	28 (46%)	
FRC Z Score	-0.65 (0.67)	-0.83 (0.70)	0.18 (-0.15; 0.50)
FRC % predicted	*88.2 (11.4)*	*83.4 (13.8)*	*4.9 (-1.3; 11.0)*
RV Z Score	0.18 (0.73)	0.10 (0.72)	0.08 (-0.27; 0.44)
RV % predicted	*104.4 (19.9)*	*102.7 (20.4)*	*1.8 (-8.1; 11.7)*
TLC Z Score	-0.50 (0.81)	-0.59 (0.90)	0.09 (-0.32; 0.50)
TLC % predicted	*95.1 (9.0)*	*92.9 (10.8)*	*2.3 (-2.6; 7.1)*
RV/TLC Z Score	0.56 (0.93)	0.54 (1.06)	0.26 (-0.45; 5.02)
RV/TLC % predicted	*109.7 (17.3)*	*110.8 (21.5)*	*-1.1 (-10.5; 8.3)*

Unless stated otherwise, results presented as mean (SD). All lung volume results expressed as percent predicted and Z Scores based on reference equations by Rosenthal *et al*[98]

In summary, results from the inter-centre comparison demonstrated that with strict adherence to a standardised protocol and prospective over-reading (see section 6.8), no bias in plethysmographic data collected in different laboratories in the UK and USA occurred, thus enabling data to be combined and used to compare to:

- published reference data:[97,98] section 6.7.1
- healthy White children of the same age: section 6.7.3
- children with SCD: section 6.11.3

6.7 Reference data

Plethysmographic lung volume data were collated from 68 healthy Black children (section 6.6) and 115 healthy White children aged 6 to12 years (Table 6-3). Significant differences in height, weight and BMI Z Scores were observed, with Black children being taller, and heavier than their White peers of same sex and age (Table 6-3).

Table 6-3: Comparison of demographics in Black and White children in whom plethysmography measurements were obtained.

	Black	White	Mean Diff (Black-White) (95%CI)
n (% male)	68 (46%)	115 (45%)	
Age (years)	10.0 (1.5)	8.9 (1.7)	1.0 (0.6; 1.5)***
Height Z Score	0.6 (1.1)	0.3 (1.0)	0.3 (0.0; 0.7)*
Weight Z Score	0.8 (1.1)	0.3 (0.9)	0.6 (0.2; 0.8)***
BMI Z Score	0.7 (1.0)	0.2 (0.8)	0.6 (0.2; 0.7)**

Unless stated otherwise, results presented as mean (SD) *p<0.05, **p<0.01, ***p<0.001
Demographics were expressed as Z Scores according to the CDC reference equations[131]

The absolute partitioned lung volumes (FRC, RV, TLC, and RV/TLC) versus height are shown in Figure 6-1. Since there is a strong relationship between FRC, RV and TLC with height and age, and a decrease in RV/TLC with height and age, and known sex differences,[147] all lung volume outcomes needed to be adjusted for these factors to enable comparison between groups of healthy Black and White children, and between healthy children and children with lung disease. Two paediatric reference equations were used to adjust for height and sex: Those by Zapletal[97] expressed results as percent predicted, whereas those by Rosenthal[98] expressed results as both percent predicted and Z scores. For the purpose of comparing the two equations, results will be expressed as % predicted.

NB: The original equations by Rosenthal did not include a calculation for RV/TLC, however personal correspondence provided us with the equations subsequently used by this group.[148]

The following section will first compare the two reference equations in healthy children (section 6.7.1), then investigate their suitability in healthy Black and White children (section 6.7.3). Quality control and repeatability measures are investigated (section 6.8) prior to developing recommendations for interpreting plethysmographic lung volumes (section 6.10) and applying them in children with SCD (section 6.11).

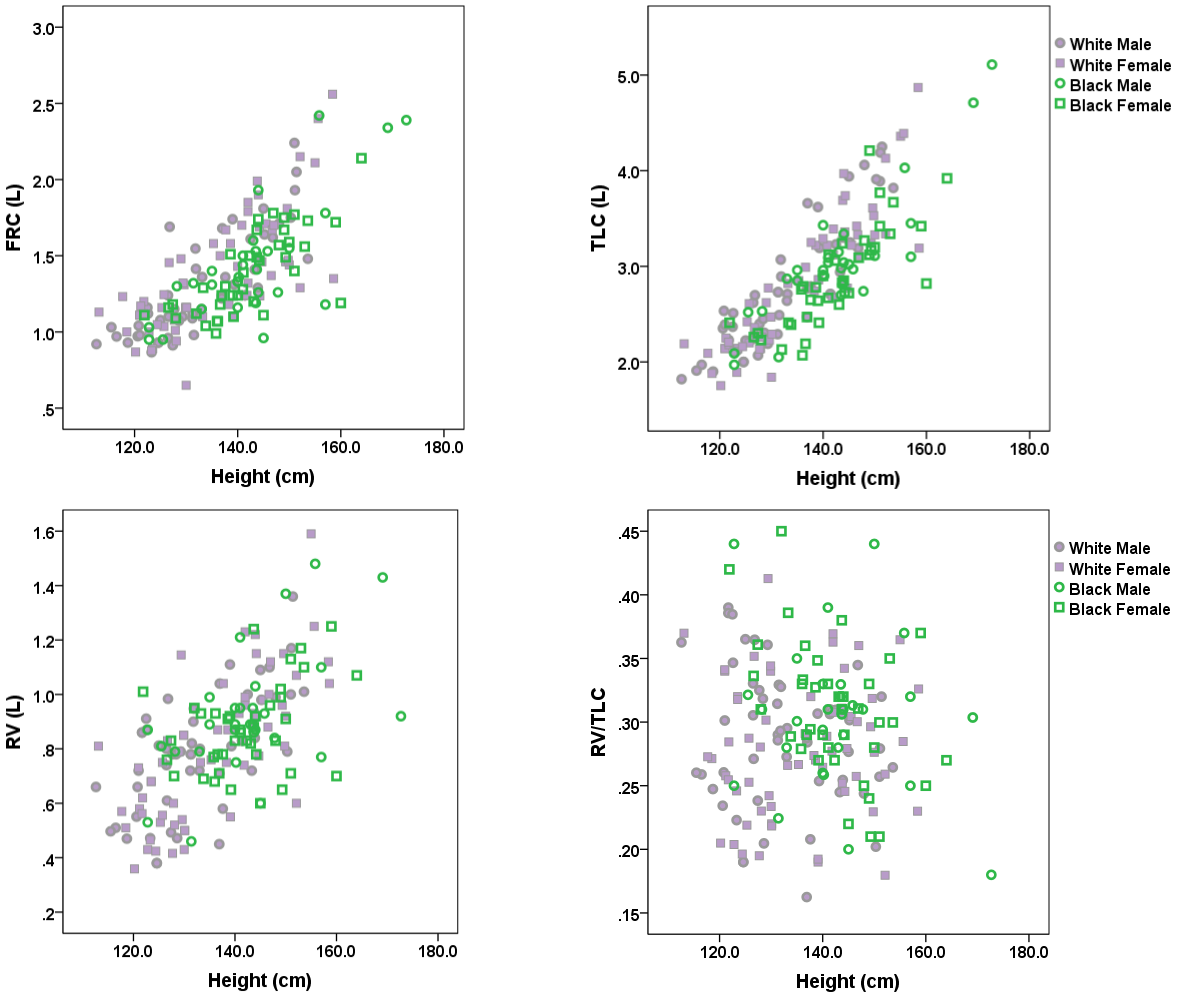

Figure 6-1: Absolute lung volumes against height in healthy children
Results based om 68 Black children and 115 White children aged 6 to 12 years.
With the exception of RV, significant ethnic differences were observed: White children had greater values for FRC and TLC, and lower RV/TLC value

6.7.1. Comparison of reference equations

Results from 183 Healthy Black and White children were available with which to compare the two paediatric plethysmography equations (although all subsequent analyses were performed separately for each ethnic group). Bland and Altman comparisons between the two equations revealed good agreement for FRC (mean difference (Zapletal-Rosenthal): 2.1% (95% LA -8.5; 12.7), however larger discrepancies for other lung volumes were observed: Mean difference (95% LA): 7.5 (-8.6; 23.7), -5.1 (-12.2; 2.1) and 13.3 (-0.2; 26.8) for, RV, TLC and RV/TLC respectively (Table 6-4 and Figure 6-2).

Table 6-4: Bland & Altman comparison of 2 plethysmographic reference equations

	Mean Difference (Zap - Ros)	95% Limits of Agreement
FRC % predicted	2.1	-8.5; 12.7
RV % predicted	7.5	-8.6; 23.7
TLC % predicted	-5.1	-12.2; 2.1
RV/TLC % predicted	13.3	-0.2; 26.8

Zap = Zapletal[97], Ros = Rosenthal[98]

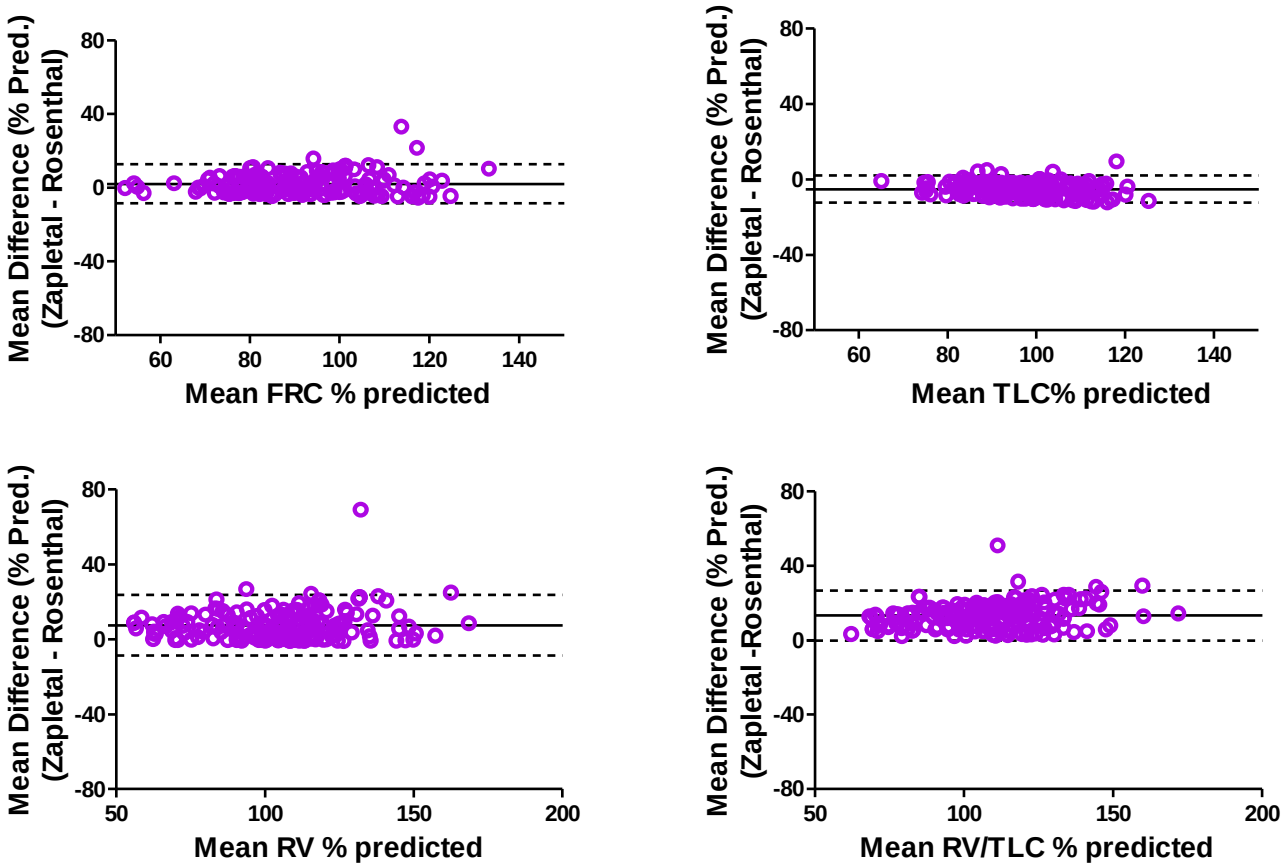

Figure 6-2: Bland & Altman plots for lung volume outcomes expressed as % predicted using two paediatric reference equations.
Results based on 183 healthy children aged 6 to 12 years.
Legend: Reference equations by Zapletal[97] and Rosenthal.[98] Solid line denotes the mean difference; dashed line denotes the 95% limits of agreement. Small mean differences in FRC were observed, however larger discrepancies in other outcomes mean that these two equations are not interchangeable.

6.7.2 Interpretation according to different reference equations

In this study, we found significant differences in % predicted of lung volumes, particularly with respect to RV and hence the RV/TLC ratio. Interpretation of lung volumes can therefore vary widely depending on which equation is applied, potentially resulting in significant clinical implications. Figure 6-3 shows the changes in predicted values for lung volume outcomes in a boy of "normal" height (i.e. a height Z Score of zero[131]) for the two equations.

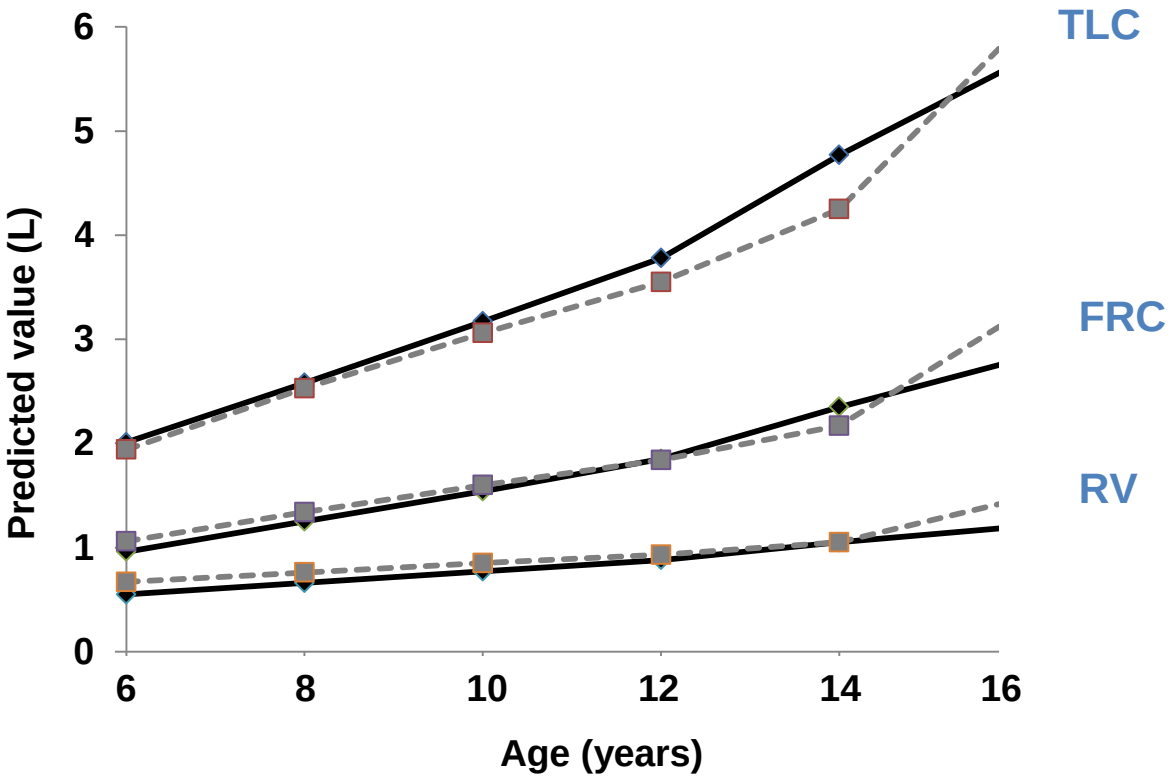

Figure 6-3: The changes in lung volumes predicted values in a boy with a height Z Score of zero.
Legend: Solid black lines are predicted values calculated by Zapletal[97] and dashed grey lines are calculated by Rosenthal[98]
Predicted values differ for all outcomes at all ages with significant cross-over at the start of puberty (~14 years where the Rosenthal equation switches at 162.5cm to post-puberty equations)

Up to about the age of ~13 years (i.e. pre-puberty) the equations are relatively similar, but some cross-over is apparent. At 165.5cm the Rosenthal[98] equations switch to new equations which supposedly take pubertal changes into account. The impact of puberty was not investigated in this study due to the restricted age limit (primary school children aged 6 to 12 years).

6.7.2.1 Case studies: Interpretation according to different reference data

Two case studies of healthy children are reviewed in Table 6-5. Both children were healthy but interpretation differed depending on the reference data applied. In both case studies, TLC % predicted was similar, and within the normal range (i.e. no evidence of restrictive lung disease), however FRC % predicted was 10% greater, and RV % predicted dramatically lower when calculated using the Zapletal equations.

Table 6-5: Case Studies: Difference in lung volume interpretation.

	Case Study A		**Case Study B**	
Demographics:	White Male, 6.3y, Height Z Score: -0.44		Black Female, 7.8y, Height Z Score: -0.79	
Lung Volumes % Predicted:	Rosenthal[98]:	Zapletal[97]	Rosenthal[98]:	Zapletal[97]
• **FRC % Predicted**	98	108	95	105
• **RV % Predicted**	75	92	**150**	**175**
• **TLC % Predicted**	100	96	112	112
• **RV/TLC % Predicted**	74	97	**130**	**160**

A difference of 10% predicted in FRC potentially has clinical implications, but this did not alter the interpretation in these examples. The differences in RV however, and consequently, differences in RV/TLC, potentially changed the interpretation of these two cases depending on the equation applied.

- Case study A: All results were within normal limits according to Zapletal equations.[97] When expressing results as % predicted using Rosenthal equations,[98] a significantly reduced RV (and consequently RV/TLC) was observed. These results were not consistent with a "healthy child"
- Case study B: When results were expressed according to Zapletal equations[97] the RV and RV/TLC were severely elevated consistent with obstructive lung disease. The Rosenthal equations[98] also presented an elevation in RV and RV/TLC but only mildly.

The larger discrepancies observed in case study B are probably due to ethnic differences which have not been accounted for in either of the reference equations. The impact of ethnicity is investigated in section 6.7.3.

6.7.2.2 Case study: The impact of height on interpretation

The influence of height was investigated in a ten year old White boy with restrictive lung disease in whom results were adjusted to represent a child short for age (-2 Z Scores), "normal" height for age (0 Z Scores) or tall for age (+2 Z Scores)[131] (Table 6-6).

At a "normal" height (0 Z Scores) differences between the two equations were minimal. Larger discrepancies however, were observed in the growth restricted (-2 Z Scores) example where FRC and RV/TLC were 10% higher, and RV 17% higher when interpreted using the Zapletal,[97] suggesting that Zapletal under-estimates predicted values (thus inflates % predicted values) in comparison to the Rosenthal equations.[98] These results have important clinical implications as growth restriction is common in lung disease.[136] In addition, the scenario of a "tall-for-age" child (commonly observed in healthy Black children compared to their White peers (Table 6-3)) differences in calculated RV and TLC % predicted differed by ~6% in opposite directions resulting in large discrepancies in RV/TLC % predicted, which also has important clinical implications.

In summary, interpretation of lung volumes can vary widely depending on which equation is applied. The two equations examined are not interchangeable and caution should be applied when reviewing results from different laboratories which may have used different reference equations for interpretation. Zapletal equations[97] were developed over 40 years ago using equipment which is now obsolete and were based on a relatively small sample size (173 children), and appear to generate greater extremes in the interpretation. The Rosenthal equations[98] were developed more recently (1993) with modern equipment and a larger sample size (772 children), however age was not taken into consideration in the reference equations and the impact of height has potential clinical implications (discussed further in the main discussion, Chapter 9). The following sections review the applications of these equations in healthy Black children and, after further analysis of QC and repeatability, recommendations for applying and interpreting lung volume reference data are developed in section 6.10.

Table 6-6: Case Study: The impact of height on predicted value for lung volumes.

	Height: -2 Z Scores		Height: 0 Z Scores		Height: +2 Z Scores	
	Rosenthal	**Zapletal**	**Rosenthal**	**Zapletal**	**Rosenthal**	**Zapletal**
FRC % Pred.	115	125	86	87	79	78
RV % Pred.	107	124	89	87	84	78
TLC % Pred.	107	105	77	73	71	66
RV/TLC % Pred.	108	118	121	124	116	127

Footnote: Example based on a 10 year old boy with an FRC of 1.5L, RV of 0.8L, TLC of 2.6L and RV/TLC of 0.31.

6.7.3 Comparison of lung volumes in Black and White children

Plethysmographic lung volume data from 68 healthy Black children and 115 healthy White children aged 6 to 12 years (Table 6-3) were compared. With the exception of RV, there were significant differences between Black and White children for all lung volume outcomes, but the magnitude and direction of these differences varied markedly according to outcome and equation selected (Table 6-7 and Figure 6-4). Thus, while there were no ethnic differences in RV by either equation, FRC % predicted was 6-9 % lower in Black children, whereas % predicted RV/TLC ratio was ~ 12% higher among Black children.

Table 6-7:Comparison of Plethysmographic outcomes according to two reference equations.

	Black children (B)	**White children (W)**	**Mean Difference (95%CI; B-W)**
n (% male)	68 (43%)	115 (45%)	
By Zapletal:[97]			
FRC % predicted	86.6 (12.3)	95.1 (15.6)	-8.5 (-12.8; -4.1)***
RV % predicted	109.0 (21.8)	107.8 (24.5)	1.2 (-5.9; 8.2)
TLC % predicted	88.6 (9.2)	96.9 (10.0)	-8.2 (-11.2; -5.3)***
RV/TLC % predicted	122.5 (21.2)	110.8 (20.1)	11.8 (5.5; 18.1)***
By Rosenthal:[98]			
FRC % predicted	86.2 (12.6)	91.2 (15.4)	-5.8 (-10.1; -1.4)*
RV % predicted	103.7 (20.0)	99.0 (23.4)	4.7 (-2.0; 11.4)
TLC % predicted	94.2 (9.8)	101.7 (11.0)	-7.5 (-10.6; -4.3)***
RV/TLC % predicted	110.2 (19.0)	96.9 (19.0)	13.2 (7.5; 19.0)***

Unless stated otherwise, results presented as mean (SD), *p<0.05, **p<0.01, ***p<0.001
Footnote: Equations by Zapletal and Rosenthal are based on White children. No ethnic adjustment was applied in this table.

Even among the White children, when results were compared to those predicted by Zapletal, there was a significant bias which exceeded 5% for all outcomes except TLC. By contrast, with the exception of FRC, where mean values were 9% (0.4 Z Scores) lower than the expected mean of 100% (0 Z Scores), the Rosenthal equations provided a reasonable fit for the White children aged 6 to 12 years. Interpretation of plethysmographic lung volumes in Black children is limited when applying either equation. An interim solution is to apply an ethnic adjustment factor and interpret with caution. Interpretation strategies are investigated in the following section.

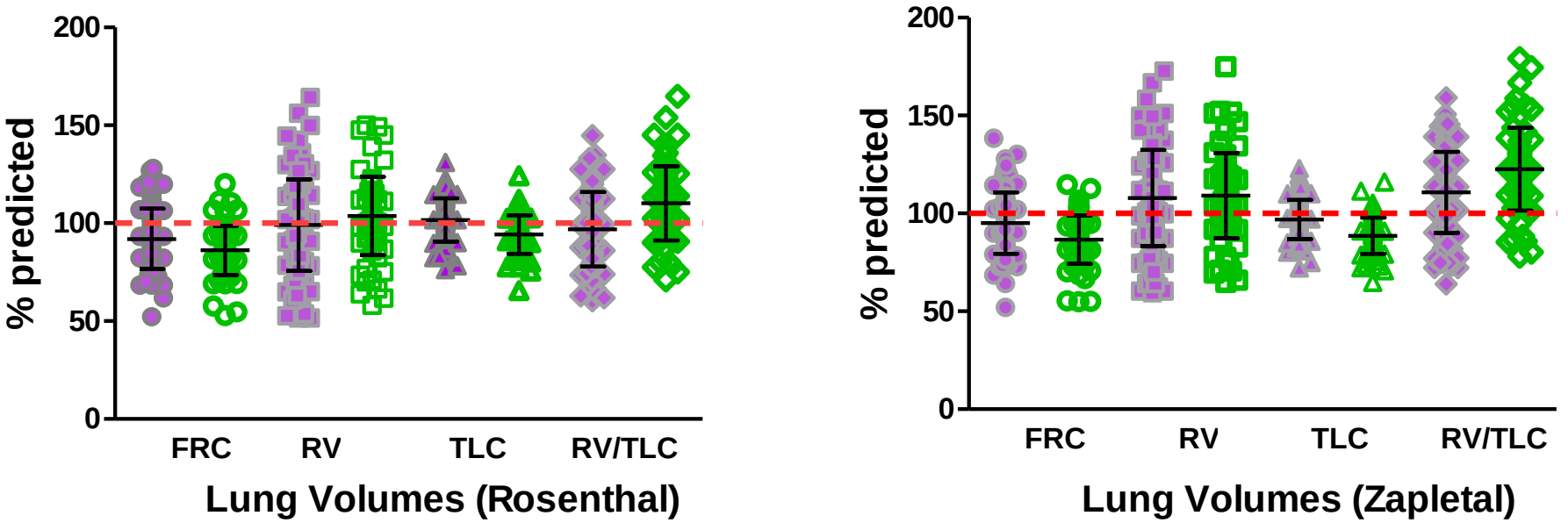

Figure 6-4: Comparison of % predicted for different lung volume outcomes for White and Black children according to two reference equations.
Legend: Reference equations by Rosenthal[98] and Zapletal.[97] Purple = White children, Green = Black children. Black lines denote the mean and SD. Dashed red line denotes 100% predicted for each outcome.
With the exception of RV which was relatively the same for White and Black children in both equations, differences between White and Black children occurred, but the magnitude and direction of the differences was dependent on the outcome and the reference data applied. Of interest, there was a large SD around the mean in RV, possibly reflecting the technical difficulty in measuring this outcome.

6.7.4 Applications of plethysmographic reference data

In the USA, the Zapletal plethysmographic equations are used most commonly,[97] whereas in the UK, the British Thoracic Society recommend reference equations by Rosenthal *et al*.[98] The Zapletal equations[97] were derived from a small sample (173) of White children aged 6 to 17 years, measured over 40 years ago, and, when compared to Rosenthal equations,[98] demonstrated large discrepancies, in particular in the extremes of height Z Scores. Rosenthal equations[98] were based on a much larger sample (772 White children aged 4 to 19 years) and developed some 20 years later, and are considered to be more appropriate of the two equations. The Rosenthal equations[98] most closely reflected the predicted results in our White population. In addition the Rosenthal equations[98] have the added advantage of calculating Z Scores (calculated as: (observed-predicted)/(SD-predicted), thus quantifying how far from the mean an individual observation is). For these reasons, reference data by Rosenthal *et al*[98] was deemed to be the more appropriate of the two. The following sections will review the feasibility of applying Rosenthal plethysmographic reference equations[98] in White and Black children.

6.7.4.1 Rosenthal equations in White children

Rosenthal plethysmographic reference equations are based on White children.[98] In this study, with the exception of FRC, the Rosenthal equations demonstrated good agreement with the healthy White children (Table 6-7). FRC was over-estimated by ~0.4Z Scores (~9% predicted) (i.e. mean Z Score in our sample was -0.4 Z (91% predicted) rather than the expected 0 Z Scores/100% predicted). This discrepancy may reflect a change in protocol during recent years, whereby subjects are no longer required to pant rapidly during airway occlusions for thoracic gas volume manoeuvres, a practice that may in the past have led to elevated resting lung volumes. This discrepancy has important implications since clinical evidence of hyperinflation or gas trapping may be missed unless this bias is taken into account, as reported previously.[16,104] In order to apply the Rosenthal equations appropriately in White children, we recommend an adjustment factor of 0.91 for FRC measures (i.e. FRC should be divided by 0.91 before applying the Rosenthal equations) (Table 6-8).

Table 6-8: Plethysmographic lung volume data and calculated limits of normality from 115 healthy White children aged 6 to 12 years.

	Mean (SD)	LLN (95%CI)	ULN (95%CI)
FRC % predicted	91.2 (15.4)	61 (**58**; 64)	121 (118; **124**)
FRC % predicted	*Adjustment factor: 0.91*	*68 (**65**; 70)*	*134 (131; **137**)*
RV % predicted	99.0 (23.4)	53 (**48**; 57)	145 (141; **149**)
TLC % predicted	101.7 (11.0)	80 (**78**; 82)	123 (121; **125**)
RV/TLC % predicted	96.9 (19.0)	60 (**56**; 63)	134 (131; **138**)
FRC Z Score	-0.4 (0.8)	-2.0 (**-2.1**; -1.8)	1.2 (1.0; **1.3**)
FRC Z Score	*Adjustment factor 0.91*	*-1.8 (**-1.9**; -1.6)*	*1.8 (1.6; **1.9**)*
RV Z Score	0.0 (0.8)	-1.6 (**-1.7**; -1.4)	1.6 (1.4; **1.7**)
TLC Z Score	0.1 (0.9)	-1.6 (**-1.8**; -1.5)	1.9 (1.7; **2.0**)
RV/TLC Z Score	-0.1 (0.9)	-1.9 (**-2.0**; -1.7)	1.7 (1.5; **1.8**)

Lung volume results corrected for sex and height using Rosenthal reference equations[98]
LLN= lower limit of normal; ULN = upper limit of normal. Based on: mean+/-1.96 SD
95% confidence intervals around the LLN and ULN were calculated from the standard error of the mean (SEM) and provide a more precise estimation of the limits of normality. Values in bold indicate the most conservative limits, that take the actual sample size of this study into account.

6.7.4.2 Interpretation of lung volumes in White children

In the current study, after adjusting FRC by dividing the absolute result by 0.91, lung volume outcomes in healthy White children centred around 0 Z Scores (100% predicted) (Table 6-8). The lower and upper limits of normality (LLN and ULN) are calculated as +/-1.96 SD, and therefore vary depending on the SD of each outcome. For example, when expressing results as % predicted, RV had the largest SD (23.4%) and therefore limits of normality were 48% predicted to 149% predicted, whereas TLC had a much smaller SD (11%), such that the limits of normality were much narrower (78% to 125% predicted). Given the relatively small sample size, the 95% confidence interval for which we can estimate these limits of normality offers a more conservative estimate of the LLN / ULN (Table 6-8, Figure 6-5).

Using Z Scores minimises the problems associated with differing SD according to different outcomes, as limits of normality (for plethysmography) are defined as +/-1.96 Z Scores. (Further explanations of limits of normality for different lung function outcomes can be found in section 1.5.1.3). In the current study the SD around the mean was <1 Z Score for all outcomes, hence minor adjustments to the limits of normality were made to account for these slight differences (Table 6-8, Figure 6-6).

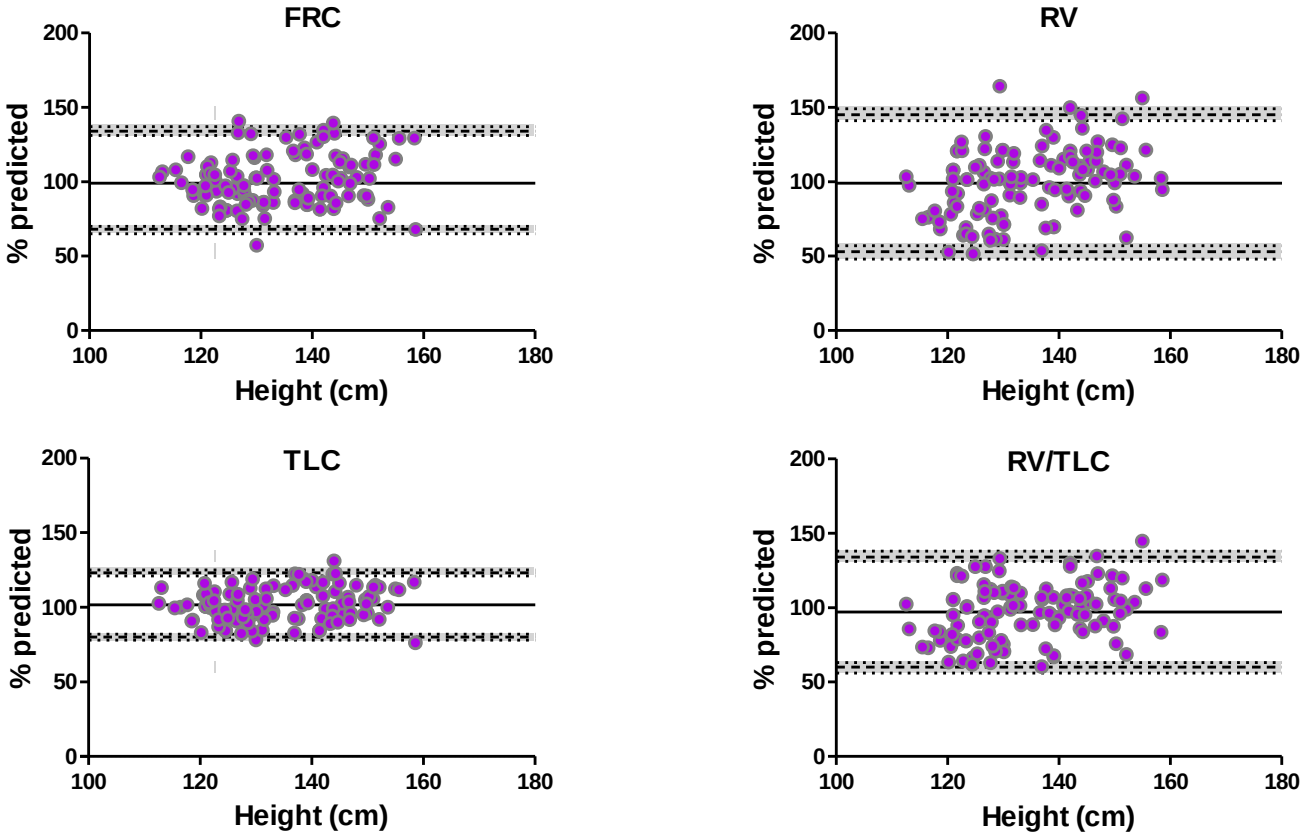

Figure 6-5: Lung Volume outcomes and calculated limits of normality presented as % predicted according to Rosenthal equations, in healthy White children.

Legend: Limits of normality calculated as +/-1.96 SD are indicated as the dashed lines, grey shaded area indicates the 95%CI around the limits of normality.
NB: FRC was adjusted by 0.91, all other outcomes remain unadjusted.
Limits of normality varied for each outcome as they were dependent on the SD of each outcome. RV had a large SD around the mean, hence limits of normality (+/-1.96 SD) were relatively wide, whereas TLC had a low SD around the mean resulting in relatively narrow limits of normality.

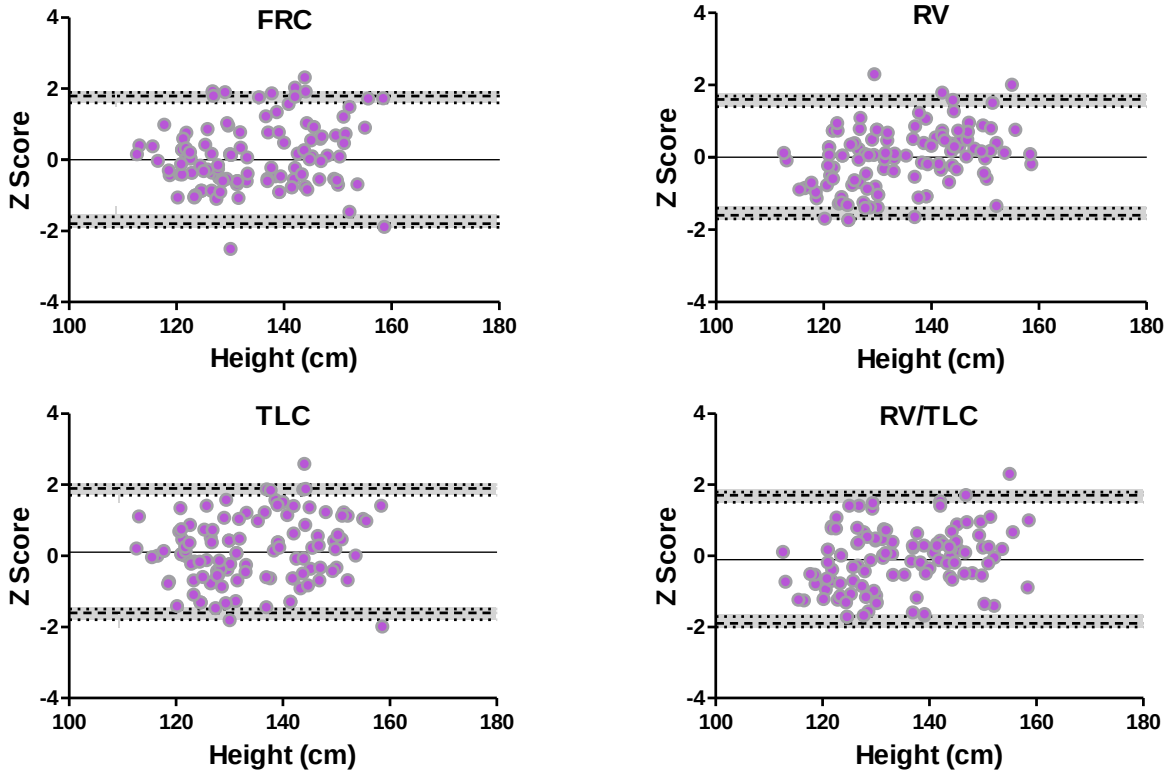

Figure 6-6: Lung Volume outcomes and calculated Limits of Normality presented as Z Scores according to Rosenthal equations, in healthy White children.

Legend: Limits of normality calculated as +/-1.96 SD are indicated as the dashed lines, grey shaded area indicates the 95%CI around the limits of normality.
NB: FRC was adjusted by 0.91, all other outcomes remain unadjusted.
With the exception of FRC (which was adjusted), all outcomes centred ~0 Z Scores. The SD around the mean however was <1 Z Score, hence the limits of normality (+/-1.96 SD) where slightly narrower than the conventional +/-1.96 Z Scores.

6.7.4.3 Rosenthal equations in Black children

Rosenthal plethysmographic reference equations,[98] based on White children were applied in 68 healthy Black children and significant discrepancies were observed: FRC and TLC were 0.7 Z Scores (14%) and 0.5 Z Scores (6%) lower in Black children than White children when predicted by these equations, whereas RV and RV/TLC respectively were 0.1 Z Scores (4%) and 0.5 Z Scores (10%) higher. In order to interpret results from Black children using these equations, the limits of normality were adjusted to take into consideration the fact that the results were not centred around 0 Z Scores or 100% predicted (Table 6-9). However, a more appropriate method of interpretation would be to apply an ethnic adjustment factor prior to applying the Rosenthal reference equations (Table 6-10, section 6.7.4.4).

Table 6-9: Plethysmographic lung volume data with no ethnic adjustment and calculated limits of normality from 68 healthy Black children aged 6 to 12 years.

	Mean (SD)	LLN (95%CI)	ULN (95%CI)
FRC % predicted	86.2 (12.6)	62 (59; 65)	111 (108; **114**)
RV % predicted	103.7 (20)	65 (**56**; 29)	143 (138; **148**)
TLC % predicted	94.2 (9.8)	75 (**73**; 77)	113 (111; 116)
RV/TLC % predicted	110.2 (19.0)	73 (69; 78)	147 (143; **152**)
FRC Z Score	-0.7 (0.6)	-1.9 (**-2.0**; -1.7)	0.5 (0.3; **0.6**)
RV Z Score	0.1 (0.7)	-1.3 (-1.5; -1.1)	1.5 (1.3; **1.7**)
TLC Z Score	-0.5 (0.8)	-2.1 (**-2.3**; -1.9)	1.1 (0.9; 1.3)
RV/TLC Z Score	0.5 (0.9)	-1.3 (-1.5; -1.0)	2.3 (2.0; **2.5**)

Lung volume results corrected for sex and height using Rosenthal reference equations[98]
LLN= lower limit of normal; ULN = upper limit of normal. Based on: mean+/-1.96SD
95% confidence intervals around the LLN and ULN were calculated from the standard error of the mean (SEM) and provide a more precise estimation of the limits of normality. Values in bold indicate the most conservative limits, that take the actual sample size of this study into account.

6.7.4.4 Interpretation of lung volumes in Black children

Ethnic adjustment factors of ~12% for TLC and RV and ~7% for FRC have been described in the ATS/ERS 2005 Interpretative strategies for lung function tests,[2] whilst many lung function software offer a blanket 12% reduction for all lung function outcomes. The current study however, suggests measured values would need to be divided by 0.86 (FRC), 1.04 (RV), 0.94 (TLC) or 1.10 (RV/TLC) prior to expressing results in relation to predicted values to adjust for the observed ethnic differences (Table 6-10).

Table 6-10: Plethysmographic lung volume data with an ethnic adjustment factor, and calculated limits of normality from 68 healthy Black children aged 6 to 12 years.

	Ethnic Adjustment	Mean (SD)	LLN (95%CI)	ULN (95%CI)
FRC % pred.	0.86	100.3 (14.6)	72 (**68**; 75)	129 (126; **133**)
RV % pred.	1.04	99.7 (19.3)	62 (57; 67)	138 (133; **142**)
TLC % pred.	0.94	100.2 (10.4)	80 (**77**; 82)	120(118; 123)
RV/TLC % pred	1.10	100.2 (17.2)	66 (62; 71)	134 (130; **138**)
FRC Z Score	0.86	0.0 (0.7)	-1.4 (**-1.6**; -1.2)	1.5 (1.3; **1.7**)
RV Z Score	1.04	0.0 (0.7)	-1.4 (-1.5; -1.2)	1.4 (1.2; **1.5**)
TLC Z Score	0.94	0.0 (0.9)	-1.7 (**-1.9**; -1.5)	1.7 (1.5; 1.9)
RV/TLC Z Score	1.10	0.0 (0.8)	-1.6 (-1.8; -1.4)	1.7 (1.5; **1.9**)

Lung volume results corrected for sex and height using Rosenthal reference equations[98]
LLN= lower limit of normal; ULN = upper limit of normal. Based on: mean+/-1.96 SD
95% confidence intervals around the LLN and ULN were calculated from the standard error of the mean (SEM) and provide a more precise estimation of the limits of normality. Values in bold indicate the most conservative limits, that take the actual sample size of this study into account.

As previously described in White children (section 6.7.4.2), use of percent predicted is associated with varying limits of normality dependent upon the SD of the outcome under investigation (Figure 6-7). Expressing the results as Z Scores simplifies the limits of agreement, albeit they have been amended slightly to account for the lower SD of each outcome (Table 6-10 and Figure 6-8).

In summary, two commonly used paediatric plethysmographic equations were evaluated and found to have marked discrepancies between them, however those by Rosenthal *et al* appeared to be the more reliable of the two. Ethnic differences in plethysmographic lung volumes were observed, and varied depending on the outcome. Reference equations based on White children are therefore not appropriate for use in Black children unless suitable adjustments both to the predicted values and limits of normality are implemented and interpretation is undertaken with caution. The following section evaluates the use of quality control criteria and repeatability measures prior to developing recommendations for the application and interpretation of plethysmographic lung volumes in children.

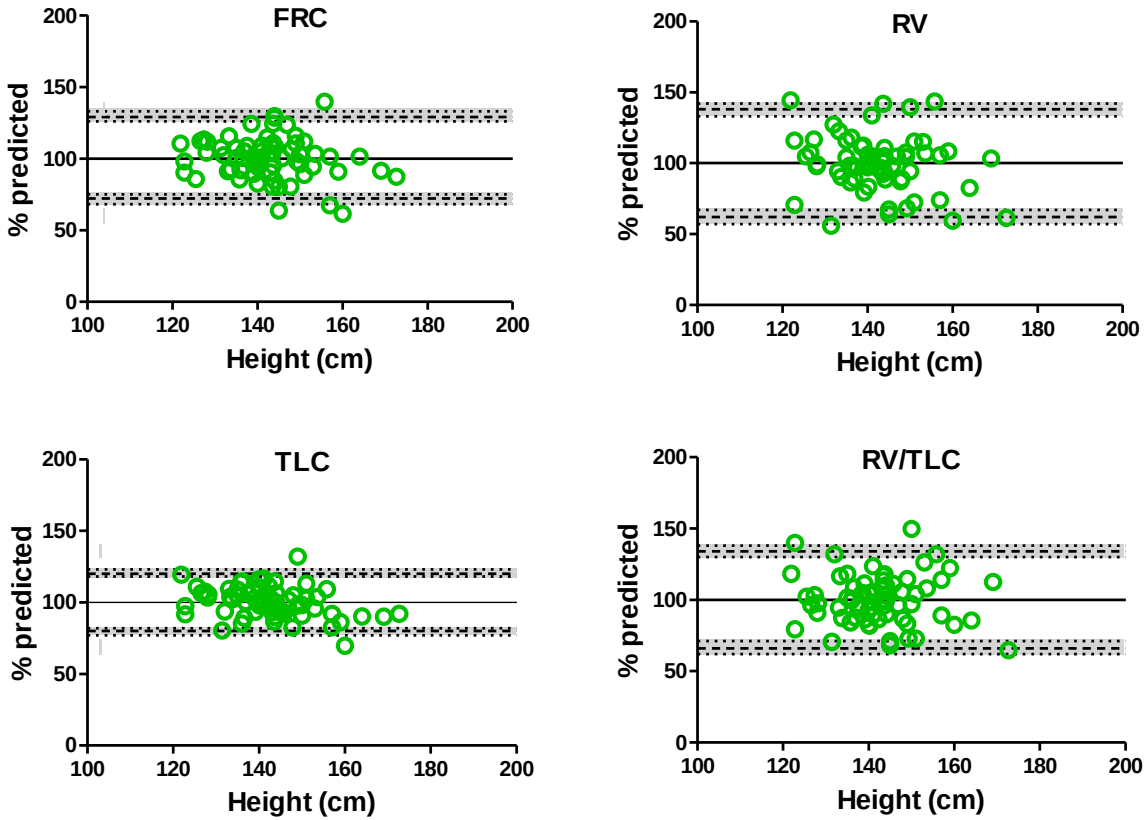

Figure 6-7: Lung Volume outcomes and calculated Limits of Normality presented as % predicted according to Rosenthal equations, in healthy Black children.

Legend: Limits of normality calculated as +/-1.96SD are indicated as the dashed lines, grey shaded area indicates the 95%CI around the limits of normality. Ethnic adjustments were made: Measured values were divided by 0.86 (FRC), 1.04 (RV), 0.94 (TLC) and 1.10 (RV/TLC) prior to expressing results
With the exception of TLC which has narrow limits of normality, wide limits of normality for all outcomes were observed (a reflection of large SD).

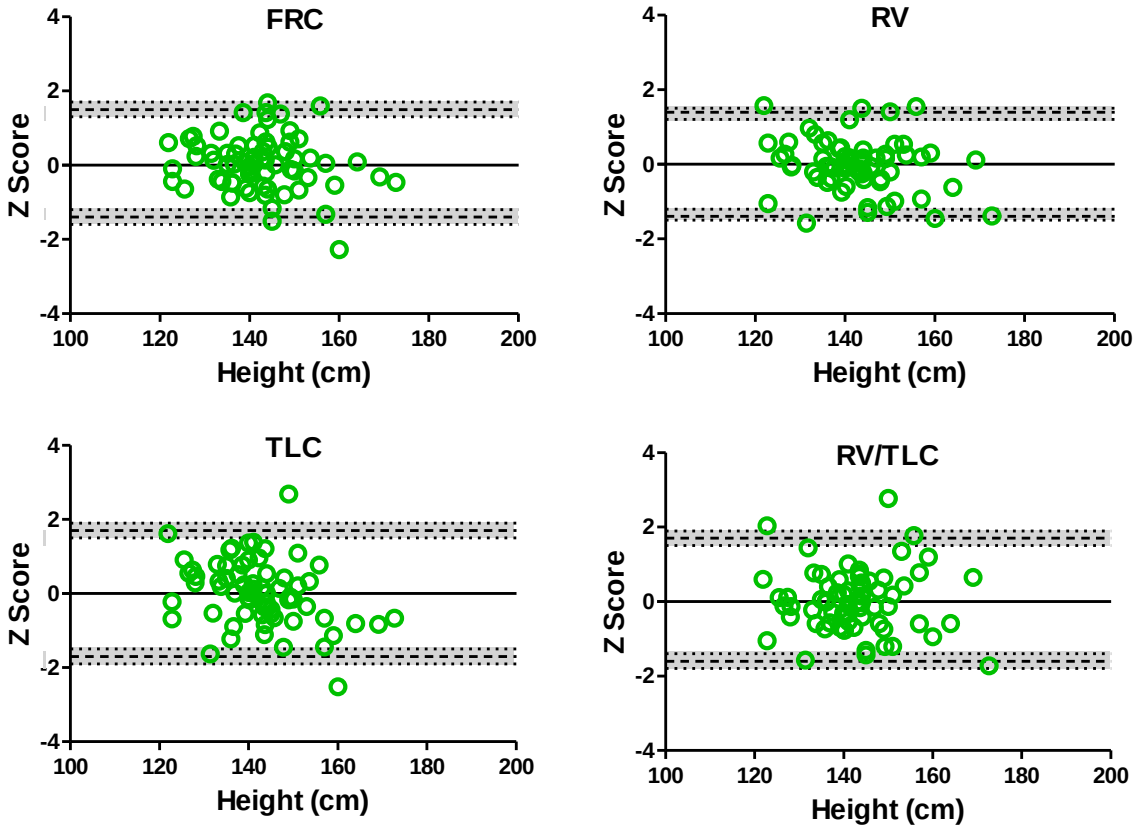

Figure 6-8: Lung Volume outcomes and calculated Limits of Normality presented as Z Scores according to Rosenthal equations, in healthy Black children.

Legend: Limits of normality calculated as +/-1.96SD are indicated as the dashed lines, grey shaded area indicates the 95%CI around the limits of normality. Ethnic adjustments were made: Measured values were divided by 0.86 (FRC), 1.04 (RV), 0.94 (TLC) and 1.10 (RV/TLC) prior to expressing results
Expressing results us adjusted Z Scores simplifies interpretation as 95% of the healthy population falls with +/-1.96 Z Scores.

6.8 Quality Control

Measurements of plethysmographic lung volumes are technically difficult to perform, and only one outcome (FRC) is directly measured, whilst the other outcomes (RV, TLC and RV/TLC) are derived from combining different outcomes (described below). Quality control assessments are therefore particularly pertinent in this effort dependent measurement.

Plethysmographic lung volumes were performed according to a standardised protocol described in chapter 2, section 2.6.6). In brief: FRC was calculated from the mean of 2-5 technically satisfactory FRC measurements (each of which consisted of at least two respiratory efforts at a breathing frequency of 30-90 breaths/min against the occlusion, with closed, super-imposable loops free from artefact/drift). RV was derived from the mean FRC minus the mean of the technically acceptable Expiratory Reserve Volume (ERV) measurements, and TLC was derived from the reported value for RV plus the largest technically acceptable Inspired Vital Capacity (IVC).

To ensure data collected met published guidelines,[94] and to document the quality of the results obtained, a plethysmographic lung volume over-read sheet was developed. Plethysmographic lung volumes were graded according to three categories:

- Performance of FRC,
- Repeatability of FRC, and
- Performance of spirometry

Each category was graded out of three, and a minimum of one for each category was required to “pass”. The over-read sheet and instructions are available in the methods, section 2.6.6.4 and the scoring table can be seen in (Table 6-11).

6.8.2 Over-read score

A random sample of lung volume data from 60 healthy Black children (mean (SD) age 10.0 (1.4) years)), 60 healthy White children (mean (SD) age 9.3 years (1.8)) and 60 children with SCD (mean (SD) age 9.9 (1.7)) were reviewed. A maximum of five FRC measures per child were reviewed. None of the data reviewed were failures (i.e. <1 acceptable FRC measurement).

Table 6-11: Quality Control scoring system for plethysmographic lung volumes

	Performance of FRC $_{(pleth)}$ trial:	
1.1	≥ 3 technically acceptable trials *(i.e. ≥2 respiratory efforts against the occlusion, BF 30-90 breaths/min)*	**3**
1.2	2 technically acceptable trials	**2**
1.3	1 technically acceptable trial	**1**
1.4	< 1 technically acceptable trials and/or breathing frequency outside recommended range*	**FAIL**
	Repeatability of FRC $_{(pleth)}$ trials:	
2.1	≥3 FRC values within 5% or 100mls	**3**
2.2	3 FRC values within 10%	**2**
2.3	2 FRC values within 5%	**2**
2.4	2 FRC values within 10%	**1**
	Performance of Spirometry measurement: **IVC within 85% of previously recorded FVC AND…..**	
3.1	Mean of 3 technically acceptable ERV's	**3**
3.2	Mean of 2 technically acceptable ERV's	**2**
3.3	Single ERV measurement (compatible with previous FVC)	**1**
3.4	No technically acceptable VC measurement	**0**

*High breathing frequencies may be associated with hyperventilation and the subsequent elevation of FRC. Results with increased breathing frequencies therefore failed QC.

Table 6-12: Lung Volume over-read scores in health and SCD.

	FRC performance score:			**FRC Repeatability score:**				**Spirometry score:**				**Overall score:**						
	Frequency (%) achieving:			Frequency (%) achieving:				Frequency (%) achieving:				Frequency (%) achieving:						
	1	2	3	0	1	2	3	0	1	2	3	3	4	5	6	7	8	9
Healthy Black	0	22	78	3	13	53	30	0	10	38	52	2	2	12	26	7	23	28
Healthy White	0	38	62	0	10	47	43	0	7	28	65	0	2	10	13	5	30	40
SCD	2	43	55	2	13	55	30	0	15	33	52	0	3	17	20	12	28	20

Results based on 60 healthy Black children, 60 healthy White children and 60 children with SCD aged 6 to 12 years.

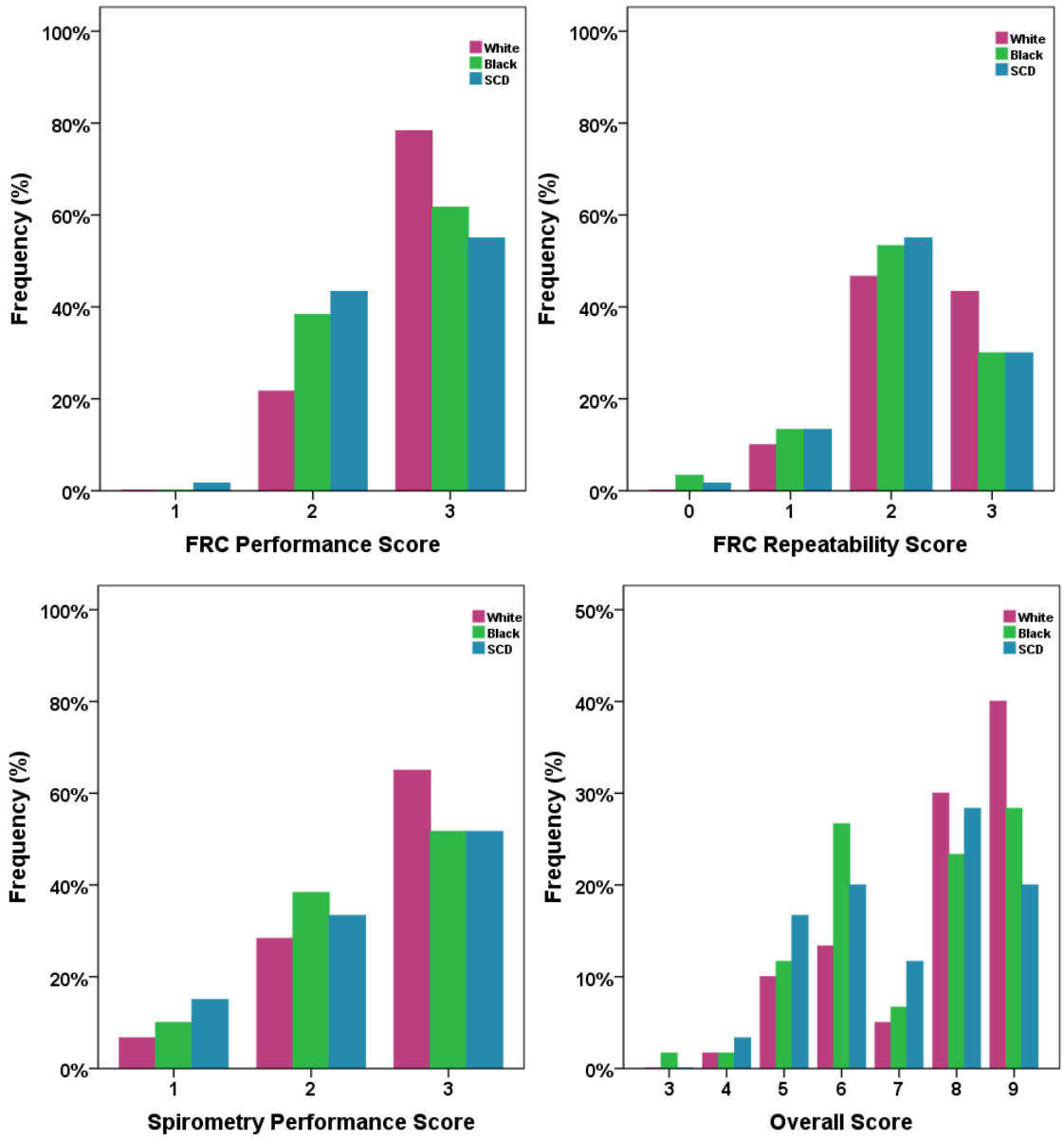

Figure 6-9: Frequency plots of lung volume over-read scores.
Results based on 60 healthy White children, 60 healthy Black children and 60 children with SCD aged 6 to 12 years.
The majority (>80%) of children reviewed achieved an overall score of >6. There was no difference in QC scores between health and disease.

Sixty-two percent of the White children, 78% of the Black children and 55% of the children with SCD performed three or more technically acceptable FRC measures and therefore scored a three for FRC performance. The FRC repeatability scores varied, with the majority scoring two (3 FRC measures within 10% or 2 FRC measures within 5%), whereas the majority of spirometry performance scores were three. The total over-read scores were similar in health and disease, with >80% of the children in all groups achieving 6 or more in the total score (Table 6-12 and Figure 6-9).

6.8.3 Summary of QC criteria

The QC criteria in Table 6-11, based on published recommendations, appear to be suitable for children aged 6 to 12 years undergoing these measurements. The majority of those reviewed performed two measures of FRC within 5% or three measures within 10%. However it is estimated that ~5% of children aged 6 to 12 are unable/refuse to perform an FRC measurement. These results were therefore not included in the review. Given that absolute lung volumes in children are lower than that observed in adults, the repeatability criteria in the over-read sheet, which is based on adult data and dependent on absolute volume differences, may be too lenient for children.

6.9 Repeatability

The QC criteria included a pre-defined repeatability criterion based on adults,[94] which the majority (80%) of the children reviewed achieved. Of the four main outcomes measured in plethysmographic lung volumes (FRC, RV, TLC and RV/TLC) only FRC is measured directly (the rest are derived from other measurements), hence the within-test repeatability of these measures may be influenced by many factors. This section will review the within-test repeatability of the FRC measurement in terms of absolute differences (maximum-minimum (mL)) and percent (([highest-lowest]/highest)*100), as well as the within-test SD and CV. The influence of age will also be evaluated.

6.9.2 FRC within-test repeatability

The literature suggests an absolute maximum within-test FRC repeatability of 150mL in adults[94] and 160mL in children.[149] In the current study, the median FRC within-test repeatability was ~ 90mL or 7% in healthy children and slightly lower (80mL or 6%) in children with SCD (Table 6-13). Non-parametric tests to determine group differences (Kruskal-Wallis Tests) revealed no significant differences between the three groups. The within-test FRC SD and CV for children aged 6 to 12 years can therefore be assumed to be around 50mLs and 3.5% respectively, this is slightly lower than previously reported in adults[94] and children.[149]

Table 6-13: Within test repeatability of FRC.

	Absolute difference (mL)	Absolute % difference	SD (mL)	Coefficient of variation (%)
Healthy Black	90 (43-130)	6.9 (3.4-10.9)	50 (25-70)	3.6 (2.0-5.6)
Healthy White	95 (50-130)	6.6 (3.9-10.2)	47 (27-71)	3.5 (2.2- 5.3)
SCD	80 (50-110)	5.8 (4.1-8.9)	42 (26-64)	3.3 (2.2-5.1)

Results presented as median (inter-quartile range) Results based on 60 healthy White children, 60 healthy Black children and 60 children with SCD aged 6 to 12 years.

6.9.3 Influence of age and within-test repeatability

The literature suggests an absolute maximum within-test FRC repeatability of 150mL in adults[94] and 160 mL in children,[149] however the current study investigated younger children aged 6 to 12 years and found within-test FRC repeatability to be lower (Table 6-13). Pearson's Correlation revealed no correlations between absolute difference (mL) and age (r=0.07, p=0.3); a significant, but weak correlation between % difference and age (r = -0.17, p = 0.02); and no correlation between SD and age (r=0.09, p=0.25) or CV and age (r= -0.14, p = 0.06) (Figure 6-10). The within-test repeatability criteria defined above (SD:50 mLs or CV: 3.5%) can therefore be considered to be applicable to all children aged 6 to 12 years.

6.9.4 Summary of repeatability

FRC within-test repeatability in children aged 6 to 12 years is slightly lower than that previously quoted in the literature which may reflect the strict QC criteria applied. There was no influence of age or lung disease on FRC repeatability in this age range; however changes during puberty (not investigated) may have a more significant impact.

Due to the nature of the test, between-test repeatability and bronchodilator responsiveness was not feasible to assess in this study.

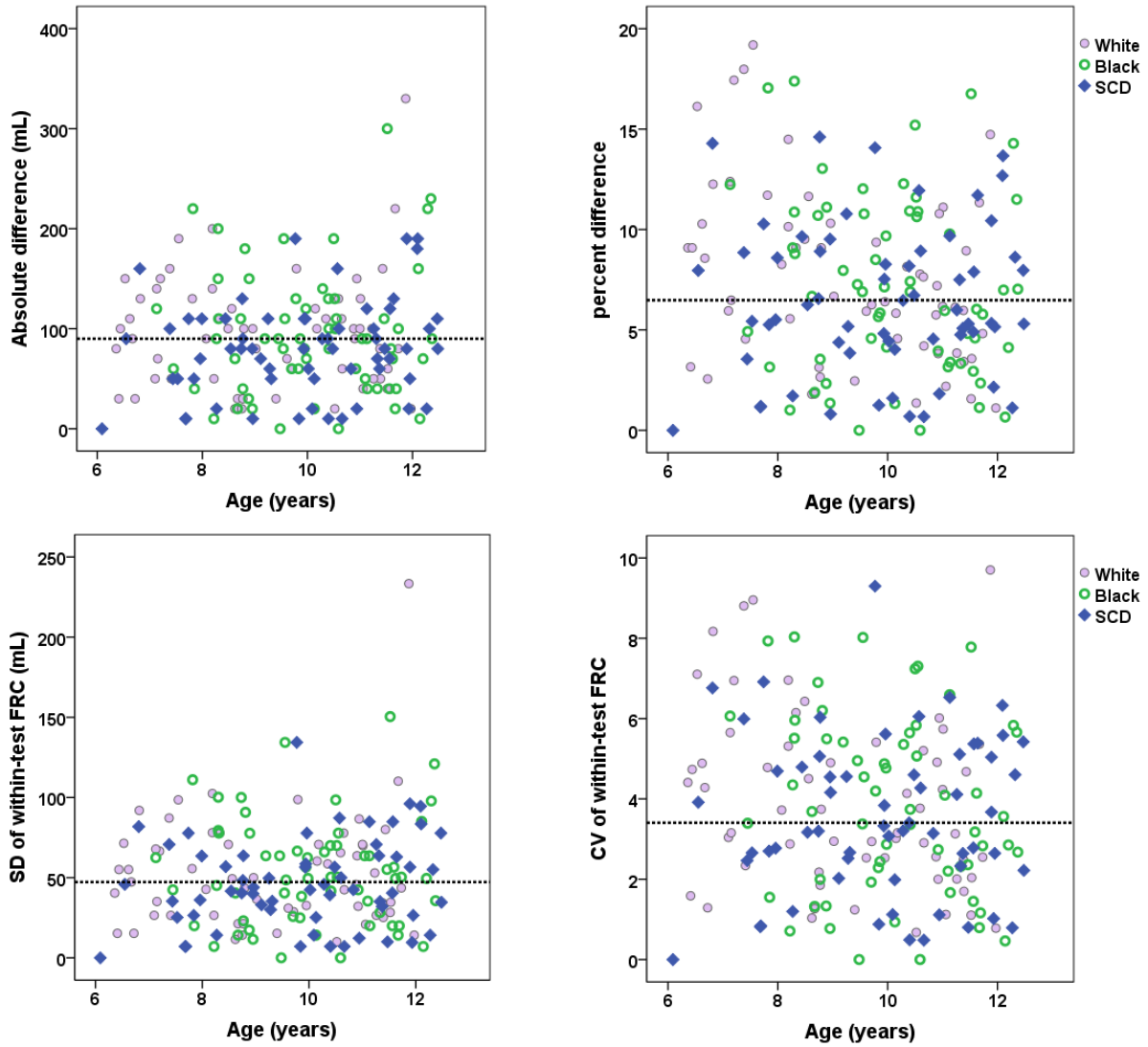

Figure 6-10: With-test repeatability of FRC in health and in SCD

Legend: Repeatability was expressed as absolute difference (mL or % difference between maximum and minimum) or as the SD (mL) or CV. Dashed line indicates the median for all children assessed.
No correlation between age and within-test FRC repeatability was observed regardless how repeatability was expressed. The median within-test FRC repeatability in healthy children and children with SCD was 90mL (absolute difference); 6.7% (percent difference); 49mL (SD) or 3.8% (CV).

6.10 Recommendations for plethysmographic lung volumes in children

Based on the results of this study the most appropriate published paediatric reference data for plethysmographic lung volumes are those by Rosenthal *et al*.[98] Ethnic adjustments for Black children should be applied (Table 6-14):

Table 6-14: Recommended Limits of normality using % predicted and Z Scores based on Rosenthal's plethysmographic reference equations

Outcome	Ethnicity	Adjustment factor	% Predicted LLN (95%CI)	% Predicted ULN (95%CI)	Z Score LLN (95%CI)	Z Score ULN (95%CI)
FRC	**Black**	0.86	72 (**68**; 75)	129 (126; **133**)	-1.4 (**-1.6**; -1.2)	1.5 (1.3; **1.7**)
RV		1.04	62 (57; 67)	138 (133; **142**)	-1.4 (-1.5; -1.2)	1.4 (1.2; **1.5**)
TLC		0.94	80 (**77**; 82)	120(118; 123)	-1.7 (**-1.9**; -1.5)	1.7 (1.5; 1.9)
RV/TLC		1.10	66 (62; 71)	134 (130; **138**)	-1.6 (-1.8; -1.4)	1.7 (1.5; **1.9**)
FRC	**White**	0.91	68 (**65**; 70)	134 (131; **137**)	-1.8 (**-1.9**; -1.6)	1.8 (1.6; **1.9**)
RV		NA	53 (49; 57)	145 (141; **149**)	-1.6 (-1.7; -1.4)	1.6 (1.4; **1.7**)
TLC		NA	80 (**78**; 82)	123 (121; 125)	-1.6 (**-1.8**; -1.5)	1.9 (1.7; 2.0)
RV/TLC		NA	60 (56; 63)	134 (131; **138**)	-1.9 (-2.0; -1.7)	1.7 (1.5; **1.8**)

LLN= lower limit of normal; ULN = upper limit of normal. NA = Not applicable.
95% confidence intervals around the LLN and ULN were calculated from the standard error of the mean (SEM) and provide a more precise estimation of the limits of normality.
Values in bold indicate the most conservative limits, that take the actual sample size of this study into account

CAUTION: While these thresholds may provide a useful guide until more appropriate reference equations can be developed, they must be interpreted with caution, given the relatively small numbers of children in the current study and can only be applied to children aged 6 to 12 years. Furthermore, it is recommended that all results undergo a QC over-reading process (Table 6-11), with good quality data scoring >6. Within-test repeatability for children aged 6-12 years should be within 90mL (max-min) or 7% (this equates to a CV of 3.5%, or a SD of 50 mL).

6.11 Applications in disease

The previous sections have described and validated a suitable method for applying a QC over-read system to plethysmographic lung volume measurements, defined within-test repeatability and suggested adjustment factors that allow published reference data to be applied in Black or White children aged 6 to 12 years. The following section will investigate the use of plethysmographic lung volume measurements in children with SCD, and compare results to healthy Black children.

6.11.2 Subjects with SCD

Lung volume data were collected in children with SCD recruited as part of the SAC study (described in Chapter 2, section 2.3.1). Age was limited to 6 to 12 years to match the healthy Black control group. Data were collected in three sites (one at ICH and two in the USA). One-way ANOVA analysis of the three groups revealed a significant difference in height Z Scores, but differences in weight and BMI Z Scores did not reach significance (Table 6-15).

Table 6-15: Comparison of demographics in 98 children with SCD aged 6 to 12 years from 3 different locations

	UK (ICH)	USA (St Louis)	USA (Cleveland)
n (% male)	59 (46%)	26 (67%)	13 (69%)
Age (years)	9.9 (1.8)	9.8 (1.8)	9.8 (1.8)
Height Z Score	0.0 (1.0)	0.2 (2.1)	-1.0 (1.1)*
Weight Z Score	-0.2 (1.0)	0.0 (1.3)	-0.6 (1.2)
BMI Z Score	-0.2 (1.0)	-0.1 (1.1)	0.0 (1.1)

Unless stated otherwise, results presented as mean (SD) *p<0.05
Demographics were expressed as Z Scores according to the CDC reference equations[131]

Children with SCD measured in Cleveland, USA demonstrated a significant difference in height Z Scores and the trend towards a difference in weight Z Scores. In addition, no control children were measured in this centre. Therefore children from this centre were excluded from further analysis.

In the two remaining centres (UK and St Louis, USA) there were no significant differences (mean difference (95%CI)) in age (0.2 years (-1.0; 0.7)); height Z Score (0.2 Z (-0.4; 0.9)); weight Z Score (0.3Z (-0.4; 0.7)) or BMI Z Score (0.2Z (-0.4; 0.6)). Thus data from these two centres could be combined and compared to healthy Black children from the same centres.

6.11.3 Comparison of lung volumes in SCD and healthy Black children

The 68 healthy Black children in whom reference data were investigated were compared to 85 children with SCD aged 6 to 12 years to determine if differences in demographics (Table 6-16) or plethysmographic lung volumes (Table 6-17) were observed. Despite no differences in age, children with SCD were significantly shorter and lighter than the healthy control children (Table 6-16).

Table 6-16: Comparison of demographics in 68 healthy Black children and 85 children with SCD aged 6 to 12 years.

	SCD	Healthy Black (HB)	Mean Diff (SCD-HB) (95% CI)
n (%male)	85 (52%)	68 (43%)	
Age (years)	9.8 (1.8)	10.0 (1.5)	-0.2 (-0.7; 0.4)
Height Z Score	0.1 (1.4)	0.6 (1.1)	-0.5 (-1.0; -0.1)*
Weight Z Score	-0.1 (1.1)	0.8 (1.1)	-0.9 (-1.2; -0.5)***
BMI Z Score	-0.2 (1.1)	0.7 (1.0)	-0.8 (-1.1; -0.5)***

Unless stated otherwise, results presented as mean (SD) *p<0.05, **p<0.01, ***p<0.001
Demographics were expressed as Z Scores according to the CDC reference equations[131]

With the exception of TLC, there were no statistical group differences in lung volume outcomes between children with SCD and healthy Black children (Table 6-17). The statistical group difference in TLC of -0.8 Z Scores (91% predicted) has significant clinical implications suggestive of restrictive lung disease in children with SCD. The extent and number of children with SCD in whom the TLC measurements fell outside the limits of normality are investigated further in the following sections.

Table 6-17: A comparison of plethysmographic lung volumes in 88 children with SCD and 68 healthy Black control children aged 6 to 12 years.

	SCD	Healthy Black (HB)	Mean Diff (SCD-HB) (95% CI)
FRC Z Score	0.0 (1.0)	0.0 (0.7)	0.0 (-0.3; 0.3)
RV Z Score	-0.2 (0.8)	0.0 (0.7)	-0.2 (-0.4; 0.1)
TLC Z Score	-0.8 (1.0)	0.0 (0.9)	-0.8 (-1.1; -0.5)***
RV/TLC Z Score	0.3 (1.1)	0.0 (0.8)	0.3 (0.0; 0.6)†

Unless stated otherwise, results presented as mean (SD) † p=0.05, ***p<0.001
All lung volume outcomes were expressed as Z Scores according to Rosenthal reference equations[98] and the ethnic adjustments derived in from Table 6-14.

6.11.4 Interpretation of lung volumes in children with SCD

Lung volume measurements are the gold standard for identifying restrictive lung disease, and are useful measurements in determining obstructive lung disease.[94] A TLC below the LLN is indicative of restrictive lung disease in an individual, whereas elevated RV, FRC or RV/TLC above the ULN indicates obstructive lung disease. Table 6-17 highlighted a statistically significant and clinically relevant group reduction in TLC in children with SCD compared to healthy Black children. Furthermore, the SCD group demonstrated a slight elevation in RV/TLC (p=0.05) in comparison to the healthy Black children.

Although group differences were identified, interpretation on an individual basis is dependent upon whether or not a result falls within the limits of normality (+/-1.96 SD). The number of children with SCD and the extent to which they fall outside the limits of normality are investigated in conjunction with the different methods of interpretation:

1) Direct application of reference equations (based on White children) with no ethnic adjustment
2) Using the lung function software adjustment of 12% for all outcomes (i.e. all results are divided by 0.88 prior to applying reference equations)
3) Applying ethnic adjustments recommended by the ATS/ERS[2]: 12% (0.88) for RV, TLC and RV/TLC ratio, and 6% (0.94) for FRC
4) Applying the ethnic adjustments based on the current study: FRC (0.86), RV (1.04), TLC (0.94) RV/TLC (1.10) and interpreting the results using:
    a. Mean Z Scores +/-1.96 SD based on the actual SD in the healthy Black children
    b. Z Scores and the conventional limits of normality (+/-1.96 Z Score)

The different methods of interpretation and the impact of over/under-diagnosis of lung disease is described in Table 6-18 and illustrated in the Figure 6-11, Figure 6-12, Figure 6-13 and Figure 6-14)

Table 6-18: Number of children with SCD with results outside the limits of normality depending on different interpretative strategies.

Method of Interpretation:	Number (out of 85) with results outside the limits of normality			
	↑FRC	↑RV	↓TLC	↑RV/TLC
No ethnic adjustment:	1	0	11	14
Software adjustment (12% for all outcomes):	3	5	3	27
ATS/ERS[2]: (7% for FRC, 12% for all others):	3	5	3	27
Ethnic adjustment based on current study: FRC:0.86; RV:1.04; TLC:0.94; RV/TLC:1.10				
• **Z Scores (+ adjusted limits of normality)**	4	1	30	7
• **Z Scores (+conventional limits of normality)**	3	0	6	7

The majority of children with SCD had FRC and RV measures within the normal range, and the different methods of interpretation did not significantly impact on the interpretation of FRC (Figure 6-11) whilst there was a slight difference in the numbers identified with a raised RV when applying the 12% adjustment advised by the ATS/ERS[2] and implemented by the software (Figure 6-12). When reviewing TLC results in SCD, applying the 12% adjustment (advised by the ATS/ERS[2] and implemented by the jaeger lung function equipment) resulted in the potential under-diagnosis of restrictive lung disease with just three children being identified by this method of interpretation. This compared to 11 children being identified when not applying any adjustment; six children when applying the 6% adjustment suggested by this study, and 30 children if using the ethnic adjustment and adjusted limits of normality suggested by this study (Figure 6-13). An evaluation of RV/TLC found that not applying an ethnic adjustment identified 14 children with a raised RV/TLC (consistent with obstructive lung disease). An apparent over-diagnosis of obstructive lung disease (when defined by an elevated RV/TLC) occurred when applying the 12% adjustment, however, using the ethnic adjustment suggested by the current study only seven children were found to have an elevated RV/TLC ratio.

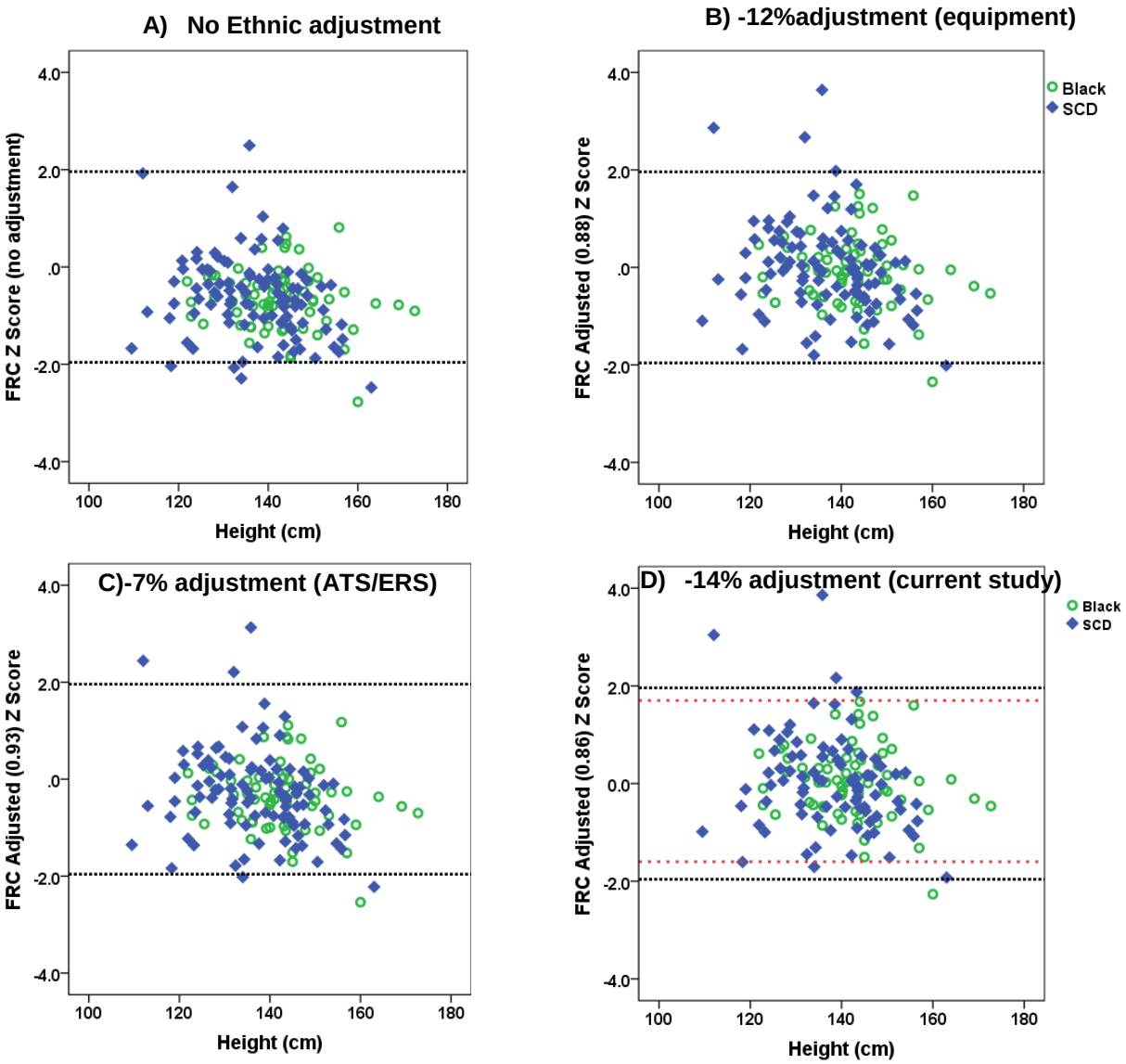

Figure 6-11: Comparison of FRC Z Scores in health and SCD.
Legend: Results based on 85 children with SCD and 68 healthy Black controls.
Black dashed line denotes the limits of normality (+/-1.96 SD). Red dotted line denotes the adjusted limits of normality based on the measured SD.
2-3 children with SCD were identified as having an elevated FRC. Interpretation did not differ significantly when applying different interpretative method

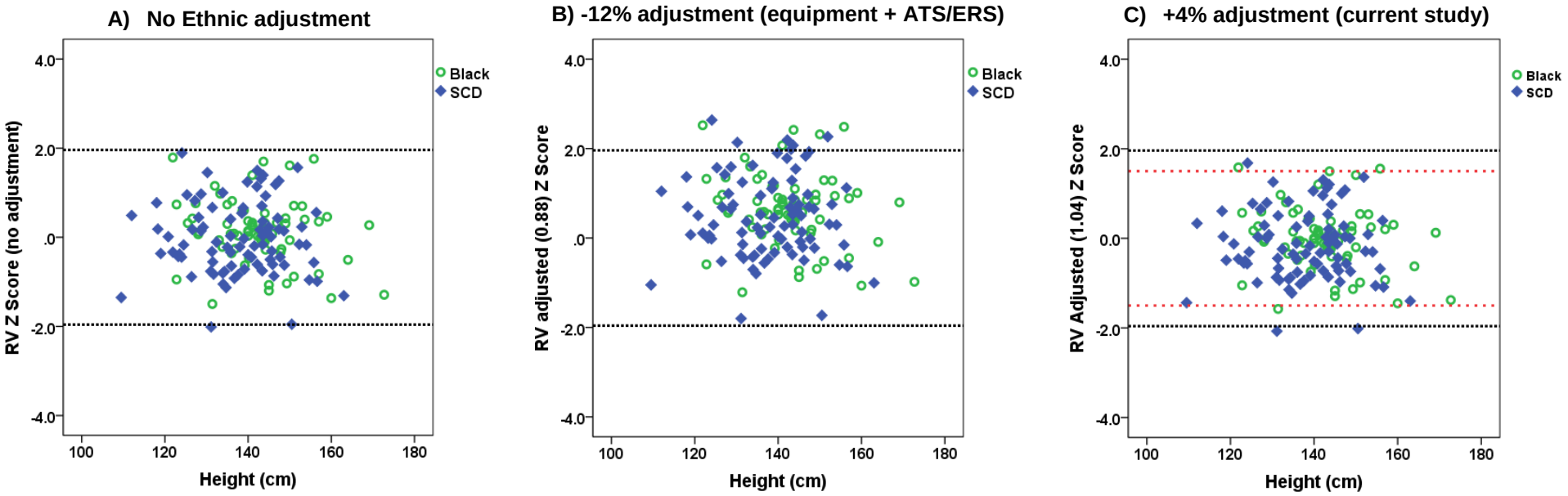

Figure 6-12: Comparison of RV Z Scores in health and SCD.
Legend: Results based on 85 children with SCD and 68 healthy Black controls.
Black dashed line denotes the limits of normality (+/-1.96 SD). Red dotted line denotes the adjusted limits of normality based on the measured SD.
When no ethnic adjustment made, or the adjustment factor derived from the current study was applied all children fell within the normal limits. Following ATS/ERS recommendations (and the option available in the equipment) of 12% adjustment resulted in 5 children with SCD having an elevated RV (although 4 healthy children also had an elevated RV). There was no significant difference in RV measurements in health or SCD.

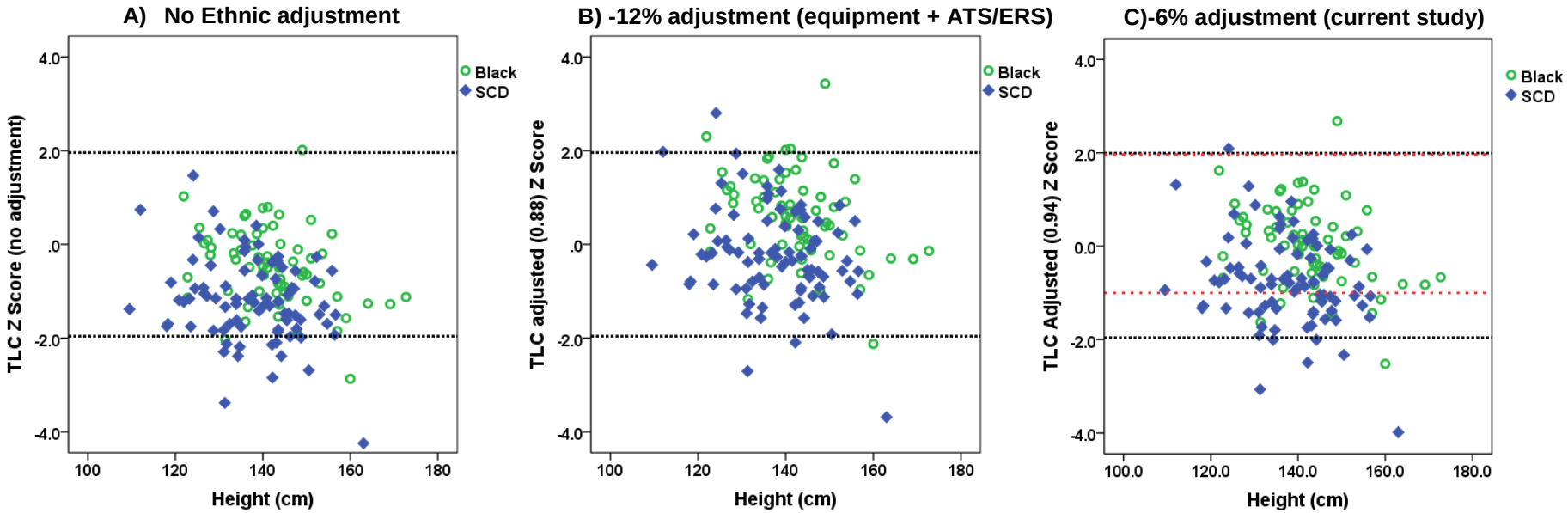

Figure 6-13: Comparison of TLC Z Scores in health and SCD.
Legend: Results based on 85 children with SCD and 68 healthy Black controls.
Black dashed line denotes the limits of normality (+/-1.96 SD). Red dotted line denotes the adjusted limits of normality based on the measured SD.
When no ethnic adjustment was made 11 children with SCD had a TLC below the lower limit of normal. Following the ATS/ERS recommendations (and the option available in the equipment) of 12% adjustment resulted in a potential under-diagnosis of restriction with just 3 children being identified. Using the ethnic adjustment based on the current study 6 children with SCD had a reduced TLC using the conventional limits of normality, and a further 24 children were identified using the adjusted limits of normality.

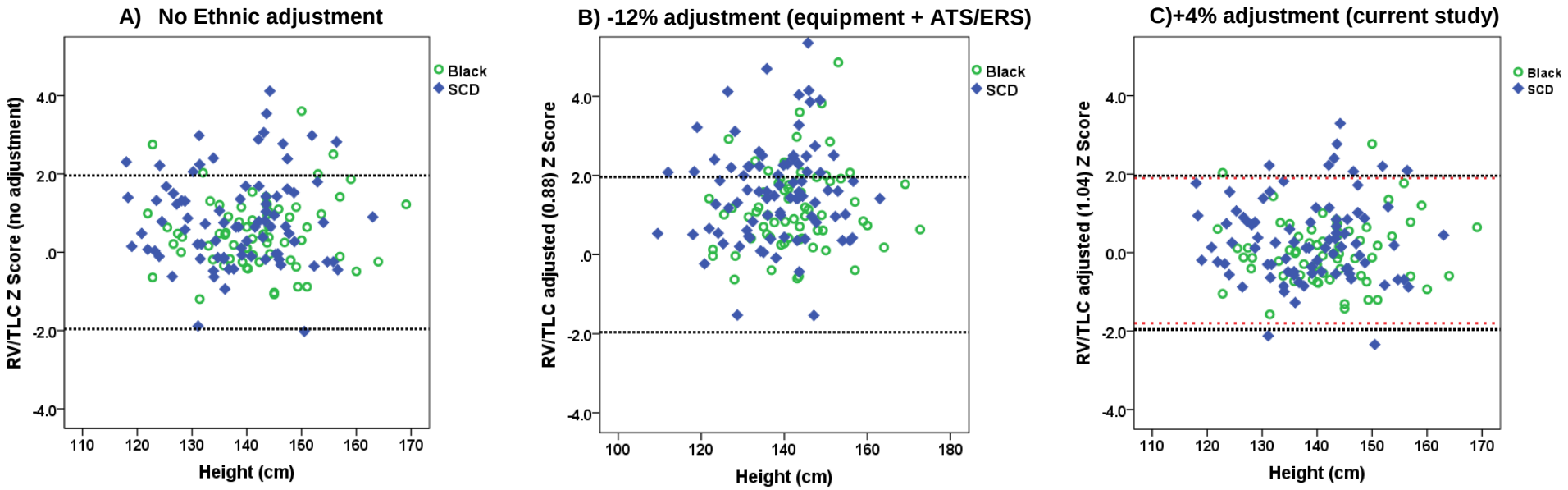

Figure 6-14: Comparison of RV/TLC Z Scores in health and SCD.
Legend: Results based on 85 children with SCD and 68 healthy Black controls.
Black dashed line denotes the limits of normality (+/-1.96 SD). Red dotted line denotes the adjusted limits of normality based on the measured SD.
When no ethnic adjustment was made 14 children with SCD had an elevated RV/TLC. Following the ATS/ERS recommendations (and the option available in the equipment) of 12% adjustment resulted in a potential over-diagnosis of obstructive lung disease with 27 children with SCD being identified with an RV/TLC above the ULN. Using the ethnic adjustment based on the current study, just 7 children with SCD had a RV/TLC above the ULN.
.

6.12 Summary

Plethysmographic lung volume measurements are the gold standard for identifying restrictive lung disease,[94] however the interpretation of these measurements in non-White children was limited due to the lack of Black specific reference data, and a dearth of paediatric specific guidelines.

Two commonly used paediatric plethysmographic equations[97,98] based on White children were evaluated and found to have marked discrepancies between them. The equations by Rosenthal seemed to be more reliable of the two; however discrepancies in FRC of ~9% in White children were still observed. In addition, significant ethnic differences were observed: FRC was 0.7 Z Scores and TLC was 0.5 Z Scores lower in Black children than White children when predicted by these equations, whereas RV and RV/TLC respectively were 0.1 Z Scores and 0.5 Z Scores higher. These equations were therefore unsuitable for use in Black children unless an adjustment was applied. Ethnic adjustments and further recommendations regarding quality control and within-test repeatability were developed, however it should be noted that these recommendations are based on a relatively small sample size of 68 healthy Black children aged 6 to 12 years. Further studies with a larger sample size and greater age range are required before definitive recommendations can be made.

Subsequent to developing recommendations for applying plethysmographic lung volumes in Black children, the measurements were performed in a group of 85 children aged 6 to 12 years with SCD. When compared with the healthy Black children of the same age, the children with SCD were found to have a significantly reduced TLC (-0.8Z Scores) and borderline significantly elevated RV/TLC (0.3Z Scores higher than the controls). Different methods of interpretation were evaluated: Failure to apply ethnic adjustments resulted in the possible over-diagnosis of raised RV/TLC, but little impact on the interpretation of the other outcomes. Applying the 12% reduction recommended by the ATS/ERS[2] and implemented by the equipment underdiagnosed restrictive lung disease (reduced TLC) and over-diagnosed RV/TLC. Applying the ethnic adjustments developed as a result of the current study appeared to be the most robust method in identifying children with lung disease. These measurements are evaluated in comparison to the other lung function assessments described in Chapters 4,5, and 7 and in relation to the clinical status in Chapter 8.

7 Spirometry

7.1 Introduction

Spirometry is the most common lung function test available and is used extensively in both clinical practice and research studies with well established guidelines for both adults,[105] and children.[1] However, since ethnic differences in lung function are known to exist,[150] accurate interpretation of spirometry in children from different ethnic backgrounds is not feasible without using appropriate reference data.[151] The most comprehensive spirometric reference data to date are the "All-Age" equations by Stanojevic *et al*,[126] which are available for White subjects aged 3 to 80 years of age whereas the only Black-specific paediatric spirometry reference data are those by Wang *et al*.[5] (NB: NHANES also provided multi-ethnic data from the age of 8 years;[107] however these were not reviewed in this thesis, since many of the children studied below the age range of these reference data). This chapter will evaluate the practicality of using either the All-Age[126] equations or the Black-specific Wang[5] equations in healthy Black children, and then apply these reference data to Black children with Sickle Cell Disease (SCD). Some of the data presented in this chapter have been published in *Pediatric Pulmonology*.[146]

7.2 Aim

The primary aims were:

i. To establish appropriate methods for interpreting spirometry data in healthy Black children and Black children with SCD
ii. To determine the extent of ethnic differences in lung function between healthy Black and White children
iii. To evaluate the extent to which spirometry detects lung disease and bronchodilator responsiveness in children with SCD

7.3 Objectives

The primary objectives were to collate spirometry data obtained from healthy children in different centres and compare them to published reference data. A secondary objective was to establish whether inter-centre differences occurred.

7.4 Hypothesis

Healthy Black children will generate different predicted values and lower limits of normal for spirometry in comparison to those defined by previously published reference data based on White children.[126]

7.5 Subjects and sample size

Spirometry data were collected in healthy Black and White children recruited into the SAC and SLIC studies (described previously in chapter 2, section 2.3). Power calculations demonstrated that spirometry measurements from 64 healthy children would enable differences equivalent to 0.5 SD (~5% predicted in FEV_1 and FVC) to be detected between the healthy children and published reference data with 90% power at the 5% significance level.

7.6 Reference data

A plethora of spirometry reference data in children were available[151] with the most comprehensive reference data to date being the All-Age equations.[126] These equations were continuous from 3 to 80 years and took into account the between-subject variability, such that an age-dependent lower-limit of normal could be defined. At the time of writing, these equations were limited to White subjects only. For Black children, the only paediatric reference data available were those by Wang *et al*.[5] These were created from the 6 cities study which included 989 Black children aged 6 to 18 years (NB: Equations from 1630 White children were also developed by the same authors). The Wang[5] equations were not continuous (i.e. there were 13 equations (one for each year (6 -18 years), for each sex and each outcome), and there was a fixed lower limit of normal which was defined as the percent predicted corresponding to the 5th percentile, which was equivalent to -1.64 SD.

The All-Age[126] and Wang[5] equations were directly compared to one another using spirometry data obtained in healthy Black children from this study. Prior to comparing the reference equations, prospective spirometry data obtained from healthy Black children at three different centres, collected using identical protocols, were compared and inter-centre comparisons were made to establish if data could be combined.

7.6.1 Inter-centre comparisons

Healthy Black children aged 6 to 12 years underwent spirometry measurements in three centres: London primary schools, The UCL Institute of Child Health respiratory laboratory (UK) and the Washington University respiratory laboratory (USA).

7.6.1.1 Demographic comparisons

Independent samples t-tests between children measured in London schools and the UK laboratory revealed no statistical differences in the demographics of these children. However, significant differences in the demographics of children measured in the USA were observed. After adjusting for age and sex using the CDC anthropometry reference equations,[131] unpaired t-tests revealed children in the UK to be significantly taller for age (mean difference (95% CI) Height Z Score: 0.8 (0.1; 1.4)) (Table 7-1).

Table 7-1: Demographics of healthy Black children in undergoing spirometry.

	School	UK lab	USA lab
n (% male)	140 (39%)	49 (38%)	25 (48%)
Age (yrs)	8.7 (1.7)	8.9 (1.5)	10.2 (1.4)
Height (cm)	136.2 (13.1)	137.9 (13.2)	140.7 (11.9)
Height Z Score	0.8 (1.0)	0.9 (1.2)	0.1 (1.4)
Weight (kg)	35.4 (12.2)	36.6 (12.4)	40.0 (11.2)
Weight Z Score	0.8 (1.0)	0.9 (1.1)	0.6 (1.0)
BMI	18.6 (3.7)	18.8 (3.6)	19.9 (3.5)
BMI Z Score	0.6 (1.0)	0.7 (1.0)	0.8 (0.8)

Results presented as mean (SD)
Z Scores for weight, height and BMI were based on CDC growth charts.[131]

7.6.1.2 Spirometry comparisons

Despite the slight somatic differences, one way ANOVA analysis of spirometric outcomes revealed no significant differences between the three centres when adjusted for height, sex and age according to ethnic specific Wang reference equations[5] (Table 7-2).

Table 7-2: Spirometry results in healthy children according to measurement site.

	School	UK Lab	USA Lab
n (% male)	140 (39%)	49 (38%)	25 (48%)
FEV_1 % Predicted	101.1 (12.4)	100.1 (11.1)	99.8 (12.8)
FVC % Predicted	104.5 (13.0)	101.5 (12.0)	105.0 (12.9)
FEV_1/FVC % predicted	97.1 (7.7)	99.1 (8.2)	95.3 (5.6)

Results presented as mean (SD)
NB: Wang reference equations[5] calculate % predicted only, Z Scores were not available.

7.6.1.3 Summary of inter-centre comparisons

Results from this study demonstrated that with strict adherence to protocol and prospective over-reading with rapid feedback (described in the methods, section 2.6.7.2) there was no bias between results collected in different laboratories in the USA and UK, or between spirometry results collected in London schools when compared to those measured in a specialised paediatric lung function laboratory.

7.6.2 Comparison of spirometry reference data

Spirometry data from 400 healthy children (214 healthy Black children (described in section 7.6.1) and 186 healthy White children previously assessed within the UK laboratory) were used to compare two spirometry reference equations and elucidate the impact of ethnicity. Black children were slightly older than their White peers. After correcting for age and sex, there was a significant difference in height, weight and BMI Z Scores with Black children being taller and heavier (Table 7-3).

Table 7-3: Demographics of healthy children undergoing spirometry measurements

	Black (B)	**White (W)**	**Mean Difference (95%CI; B –W)**
n (% male)	214 (40%)	186 (50%)	
Age (yrs)	8.9 (1.7)	8.4 (1.6)	0.5 (0.2; 0.8)*
Height Z Score	0.7 (1.1)	0.3 (0.9)	0.5 (0.2; 0.7)***
Weight Z Score	0.8 (1.0)	0.3 (0.9)	0.5 (0.3; 0.7)***
BMI Z Score	0.7 (1.0)	0.3 (0.9)	0.4 (0.2; 0.6)***

Unless stated otherwise, results presented as mean (SD), *p<0.05 ***p<0.001
Z Scores for weight, height and BMI were based on CDC growth charts.[131]

Spirometry results were expressed as percent predicted according to Wang,[5] using the Black or White equations as appropriate, and the Stanojevic All-Age[126] equations, which are based on White children. Both Wang (White-specific) and All-Age equations described the White population well, with Bland and Altman analysis revealing close agreement between the two equations (FEV_1: mean difference (95% limits of agreement): 2.1% (-3.8 to 8.0)). The Wang Black-specific equations also described the current Black population well, with both mean FEV_1 and FVC % predicted centred on 100% predicted (Figure 7-1). When compared with their White peers using the All-Age equations, results from Black children were ~15% lower for FEV_1 and ~13% lower for FVC (Table 7-4).

Table 7-4: Comparison of spirometric outcomes between healthy Black and White children according to two reference equations

	Black (B)	White (W)	Mean Difference (95%CI, B-W)
n (% male)	214 (40%)	186 (50%)	
Wang *et al*:[5]			
FEV_1 % pred.	99.9 (12.4)	104.4 (12.9)	-4.3 (-6.8; -1.8)**
FVC% pred.	103.0 (13.0)	104.4 (12.9)	-1.4 (-4.0; -1.1)
FEV_1/FVC % pred.	97.4 (7.6)	99.8 (6.9)	-2.5 (-3.9; -1.1)**
All-Age:[126]			
FEV_1 % pred.	86.6 (10.2)	102.1 (12.5)	-15.5 (-17.7;-13.3)***
FVC % pred.	90.1 (11.2)	103.5 (12.3)	-13.5 (-15.8;-11.2)***
FEV_1/FVC % pred.	95.4 (7.6)	97.7 (6.6)	-2.3 (-3.7; -0.9)**

Unless stated otherwise, results presented as mean (SD), **p<0.01, ***p<0.001
Footnote: Wang *et al* equations have ethnic specific equations and were calculated respectively.
All-Age equations are based on White subjects; no ethnic adjustment was made in this table.

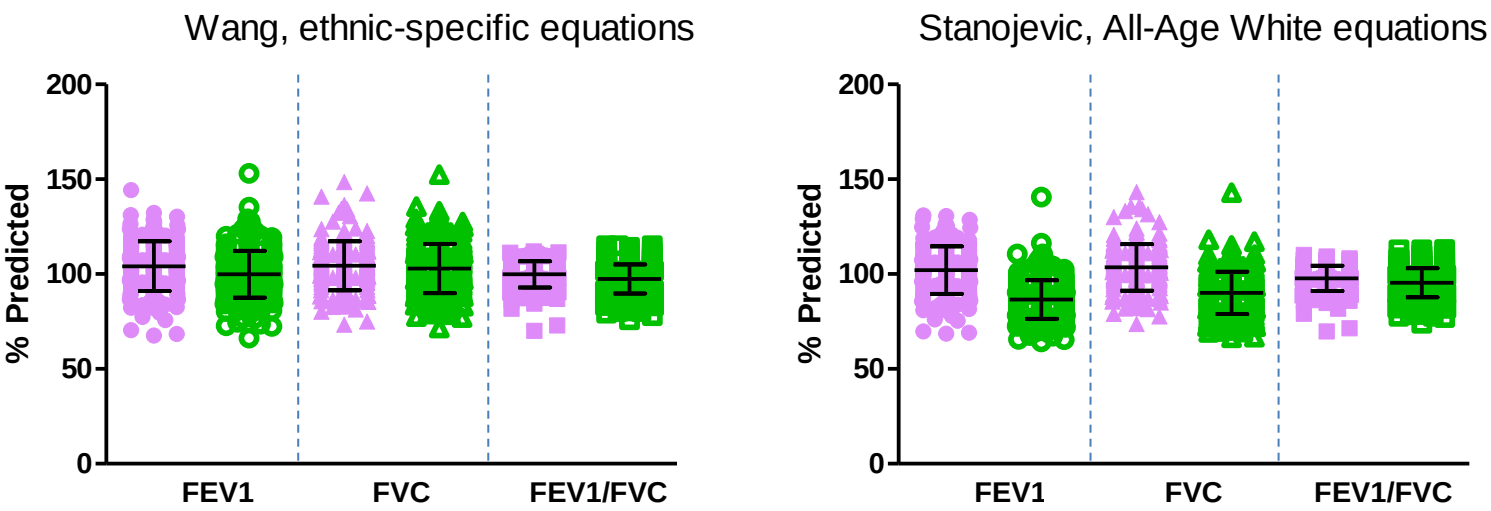

Figure 7-1: Comparison of spirometric outcomes in 400 healthy children
Results expressed as % predicted calculated by two reference equations. [5 126]

Wang equations (left panel) demonstrate good agreement for both White (purple) and Black (green)children (results approximating 100% predicted). All-Age equations demonstrate good agreement for White children, but a reduction by ~15% predicted in both FEV_1 and FVC, and hence no difference in FEV_1/FVC for healthy Black children.

7.6.2.1 Interpretation of Wang reference equations

Evaluation of the two reference equations revealed the equations by Wang *et al*[5] to be the most appropriate for interpreting spirometry results in the studied population of Black children aged 6 to 12 years. The wide SD around the mean, however, may have an impact on the expected limits of normality. Wang *et al*[5] described the lower limit of normal (LLN) to be 82-83% predicted for FEV_1 and FVC (corresponding to the 5th percentile (-1.64 SD)). However, a more conservative approach would be to define the LLN as the 2.5th percentile (-1.96 SD) as used in other lung function outcomes in children. Based on the investigated healthy population, the 95% limits of normality (i.e. +/-1.96 SD) have been calculated (Table 7-5). For example the LLN for FEV_1 in a Black child has been estimated to be 78% predicted.

Table 7-5: Limits of normality for Black and White children using % predicted based on Wang spirometry reference equations.

Outcome	Ethnicity	LLN (95%CI)	ULN (95%CI)
FEV_1	Black	80 (**78;** 80)	120 (119; 122)
FVC		82 (**80;** 83)	124 (123; 126)
FEV_1/FVC		85 (**84;** 86)	110 (109; 111)
FEV_1	White	82 (**81;** 80)	120 (124; 127)
FVC		83 (**82;** 85)	126 (124; 127)
FEV_1/FVC		89 (**88;** 89)	111 (110; 112)

LLN= lower limit of normal; ULN = upper limit of normal. NA = Not applicable.
95% confidence intervals around the LLN and ULN were calculated from the standard error of the mean (SEM) and provide a more precise estimation of the limits of normality. Values in bold indicate the most conservative limits, that take the actual sample size of this study into account.

7.6.2.2 Ethnic "adjustments" for spirometry

The Wang[5] equations may be suitable for interpreting spirometry data in Black children aged 6 to 12 years; however subjects that fall below this age range do not have suitable reference data. The observed differences in spirometric outcomes between healthy Black children and those predicted using the All-Age[126] equations (~13% for FEV_1 and ~10% for FVC (Table 7.4)) were therefore used as "ethnic adjustments." i.e. measured results from the Black children were divided by 0.87, 0.90 and 0.95 for FEV_1, FVC and FEV_1/FVC respectively before calculating predicted values according to the All-Age equations. Once these adjustment factors had been applied, Bland and Altman analysis revealed no bias across the age group studied between % predicted results calculated according to Wang *et al*[5] and those calculated from the All-Age[126] (adjusted) equations: The mean difference (95% limits of agreement) between Wang minus All-Age-adjusted was 0.3% (-9.7; 10.3) for FEV_1, 2.8% (-8.2, 13.8) for FVC and -3.7% (-10.4; 3.0) for FEV_1/FVC.

7.6.2.3 Summary of reference data comparisons

Two paediatric spirometry reference equations were reviewed. The All-Age[126] spirometry equations demonstrated excellent agreement with White children, as described previously,[32,152] but were not appropriate for Black children unless ethnic adjustments were applied. The ethnic-specific spirometry equations by Wang *et al*[5] also proved a good fit for White and Black children respectively, with mean results from our healthy children approximating 100% predicted (Table 7-4). The Wang equations are, however, limited to 13 step-wise sex-specific equations for each year of age from 6 to 18 years for each ethnic group. This step-wise approach to adjust for age potentially limits the accuracy of deriving predicted equations. Quanjer *et al* have recently demonstrated that neglecting to use decimalised age results in errors of up to 10%.[153] Furthermore extrapolating beyond these age ranges is not recommended,[125] hence these equations are not suitable for the increasing number of preschool children now undertaking such tests.[1] A temporary solution to this problem could be to use the Stanojevic equations with appropriate adjustment factors for Black children less than 6 years, albeit with caution due to the known potential problems which may occur when switching between reference equations.[151,154]

Caution should be used when interpreting spirometry using the Wang equations. The lower limit of normal (LLN) quoted by Wang *et al* as 81.3%, 81% and 90% (based on +/-1.64 SD) for FEV_1, FVC and FEV_1/FVC respectively may over diagnose abnormality, since the LLN of normal was demonstrated to be slightly lower in this study at 75.2%, 74% and 84.8%, respectively. Varying LLNs for each outcome, and age group have been described previously,[155] and can complicate interpretation. A solution to this would be to present the results as Z Scores instead of % predicted. Z Scores take into account the variability of the measurement. The LLN can be set at -1.96 Z Scores (equivalent to 2.5th percentile) or -1.64 Z Scores (equivalent to the 5th percentile, often used in adult lung function).[155] Z Scores were not available for the Wang equations, therefore all results were presented as percent predicted. The limitations observed in the Wang equations are not present in the All-Age equations, which are based on White subjects aged 3 to 80 years of age and provide smoothly changing curves to describe the transition between childhood and adulthood, whilst accounting for the age-dependent range of normal.[126] Multi-ethnic equations across all ages that allow the calculation of Z Scores are expected to be released in 2012 (www.lungfunction.org).

7.7 Applications: Spirometry in SCD

The previous sections have evaluated the available reference data for spirometry and identified which are the most appropriate for use in Black children. The following section applies these reference data to 60 children with SCD, and compares children with SCD to 214 healthy Black children.

Independent t-tests demonstrated that the children with SCD were significantly shorter and lighter for age compared with the healthy Black children (Table 7-6).

Table 7-6: Comparison of demographics in 214 healthy Black children and 60 children with SCD aged 6 to 12 years.

	Healthy Black	SCD	Mean Diff (SCD – Black) (95% CI)
n (% male)	214 (43.3%)	60 (39.3%)	
Age (years)	8.9 (1.7)	9.2 (2.1)	0.3 (-0.2; 0.8)
Height Z	0.7 (1.1)	-0.2 (1.1)	-0.9 (-1.2; -0.6)***
Weight Z	0.8 (1.0)	-0.3 (1.0)	-1.1 (-1.4; -0.8)***
BMI Z	0.7 (1.0)	-0.3 (0.9)	-0.9 (-1.2; -0.6)***

Unless stated otherwise, results presented as mean (SD) ***p<0.001
Z Scores for weight, height and BMI were based on CDC growth charts.[131]

Spirometry results were expressed as percent predicted to adjust for age, height and sex differences.[5] Group differences between SCD and health were observed (Figure 7-2): FEV_1 and FVC were significantly lower in SCD than in health; however they were reduced in equal proportions, such that there was no difference in the FEV_1/FVC ratio (Table 7-7).

Table 7-7: Comparison of spirometry obtained in 214 healthy Black children to 60 children with SCD aged 6 to 12 years.

	Healthy Black	SCD	Mean Diff (SCD – Black) (95% CI)
FVC (% pred)	103.9 (12.8)	90.1 (14.9)	-13.8 (-17.6; -10.0)***
FEV_1 (% pred)	100.7 (12.1)	88.8 (13.9)	-12.0 (-15.6; -8.4)***
FEV_1/FVC (% pred)	97.3 (7.7)	98.9 (7.4)	1.6 (-0.6; 3.8)

Unless stated otherwise, results presented as mean (SD) ***p<0.001
Z Scores for weight, height and BMI were based on CDC growth charts.[131]

Comparisons of age, height and height Z Scores[8] and spirometry results expressed as % predicted demonstrated no bias according to age or height in the age group studied. Thus, spirometry results in children who were small for age (i.e. with low height Z Scores) were no worse than in taller/older children who were “normal” height for age (Figure 7-3 and Figure 7-4).

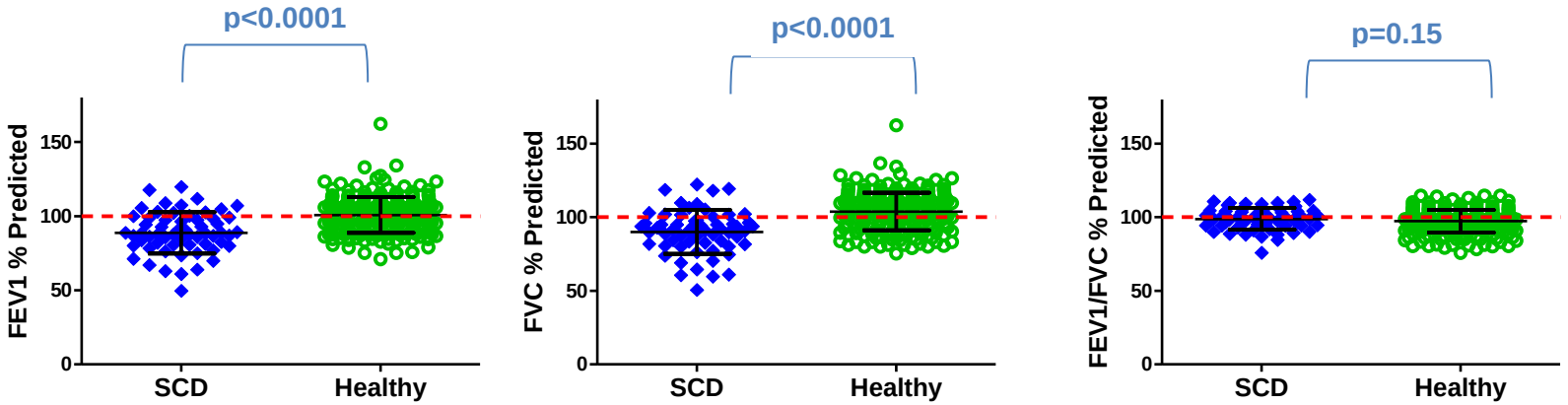

Figure 7-2: Comparison of spirometric outcomes in 214 healthy Black children and 60 children with SCD aged 6 to 12 years.
Legend: Black lines denote mean (SD). Red dashed line represents 100% predicted.
FEV_1 and FVC were significantly (p<0.0001) lower in SCD than in health; however they were reduced in equal proportions, such that there was no significant difference in the FEV_1/FVC ratio

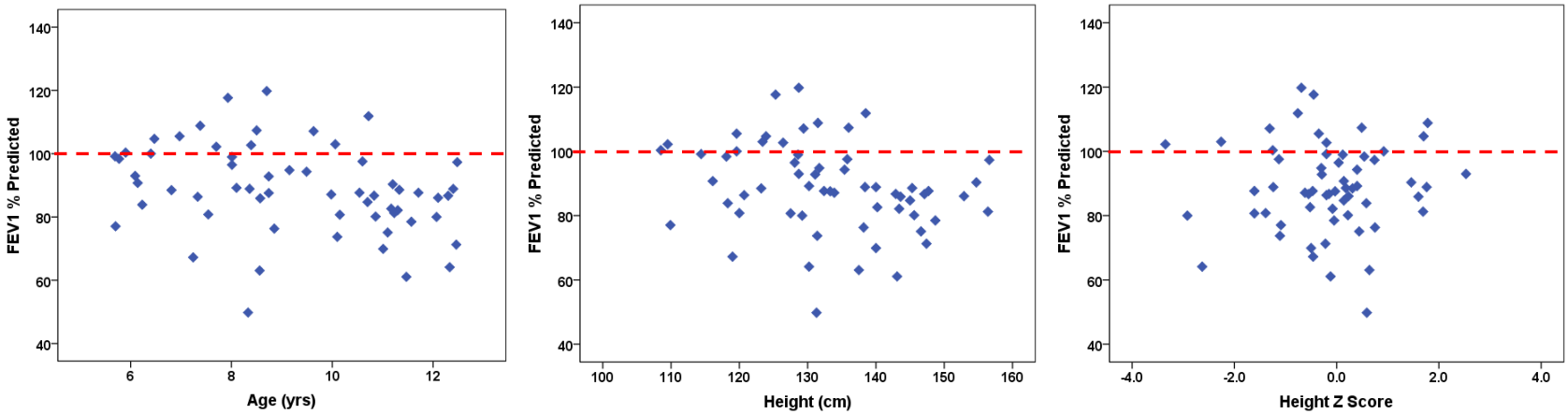

Figure 7-3: Comparison of FEV_1 % predicted and age, height and height Z Scores in 60 children with SCD.
Legend: Red dashed line represents 100% predicted.
No correlation between decreased FEV_1 percent predicted and age, height or height Z Score was observed.

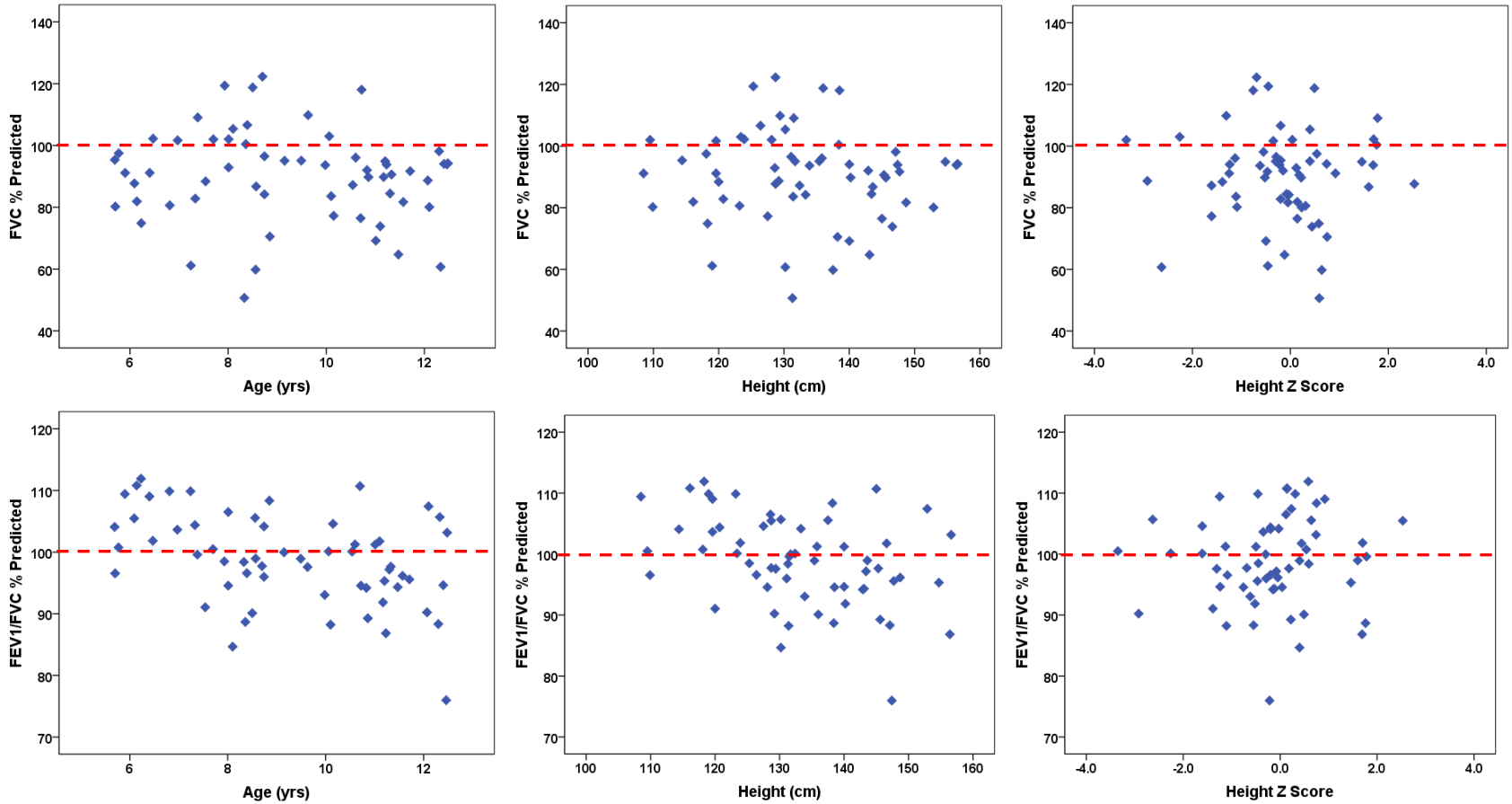

Figure 7-4: Comparison of FVC and FEV_1/FVC percent predicted and age, height and height Z Score in 60 children with SCD.
Legend: Red dashed line represents 100% predicted.
No correlation between decreased FEV_1 percent predicted and age, height or height Z Score was observed

7.7.1 Bronchodilator response (BDR)

Between-test repeatability has previously been defined as <150mL in FVC and FEV_1, with a slightly tighter criteria of <100mL if the FVC is <1.0L.[105] Several studies have demonstrated that these criteria are achievable in children,[1,105,108,111,113,114] thus between-test repeatability was not investigated further in this study. Criteria for defining a significant BDR has also been defined previously as a 12% and/or 200mL increase in FEV_1 from baseline.[2] The extent to which children with SCD responded to bronchodilators was evaluated. Prior to evaluating spirometry and BDR in children with SCD, BDR in health was examined.

7.7.1.1 Bronchodilator response in health

A subset of 50 healthy children (11 Black and 39 White) underwent BDR assessments with spirometry data as the outcome measure. The demographics and baseline spirometry of these healthy children were similar to the main group (Table 7-3 and Table 7-4), and there was no significant difference between the BDR observed in healthy White children and healthy Black children when expressed as absolute change or percent change from baseline (mean difference (Black-White), (95%CI): 42.6mL (-32.8; 118.0) and 2.0% (-2.2; 6.1) respectively). Results from these children were therefore combined and used as a reference for interpreting bronchodilator responsiveness in SCD (i.e. a clinically relevant BDR would be a change greater than that seen in health).

7.7.1.2 Comparison of bronchodilator response observed in health and SCD

Fifty-five children with SCD and 50 healthy children underwent BDR assessments. The demographics of these children were similar to the main group (Table 7-6). As observed previously, the children with SCD were significantly shorter and lighter than the healthy children (Table 7-8).

Table 7-8: Comparison of demographics in healthy children and children with SCD in whom BDR using spirometry were undertaken.

	SCD	Healthy	Mean Diff (SCD- Health) (95% CI)
n (% male)	55 (48)	50 (46)	
Ethnicity: % Black	100	80	
Age (yrs)	9.4 (2.0)	8.6 (2.2)	0.7 (-0.1; 1.6)
Height Z	-0.2 1.1)	0.3 (1.0)	-0.5 (-0.9; -0.1)*
Weight Z	-0.3 1.0)	0.2 (0.9)	-0.6 (-1.0; -0.3)***
BMI Z	-0.3 0.1)	0.2 (0.9)	-0.6 (-0.9; -0.2)***

Unless stated otherwise, results presented as mean (SD), *p<0.05,***p<0.001
Z Scores for weight, height and BMI were based on CDC growth charts.[131]

Paired t-tests revealed a statistically significant (p<0.0001) change between baseline and post bronchodilator FEV_1, in both healthy children (mean difference (95%CI): 49 (20; 80) mL) and children with SCD (mean difference (95%CI): 60 (40; 90) mL). However, when compared in terms of absolute change or percentage change from baseline, the differences observed were not clinically relevant and group differences between health and SCD were not observed (Table 7-9).

Table 7-9: Comparison of BDR expressed as absolute change, or percentage change in 50 healthy children and 55 children with SCD aged 6 to 12 years.

Change from baseline	SCD	Healthy	Mean Diff (SCD – Health) (95% CI)
FEV_1 (mL)	66 (84)	49 (11)	17 (-20; 55)
FEV_1 (%)	5.0 (7.3)	3.1 (6.0)	1.9 (-0.7; 4.5)
FVC (mL)	28 (11)	3 (15)	25 (-27; 77)
FVC (%)	2.6 (8.3)	0.5 (7.8)	2.1 (-1.0; 5.2)

Unless stated otherwise, results presented as mean (SD), No significant differences.

No significant relationship between age and BDR was observed (Figure 7-5).

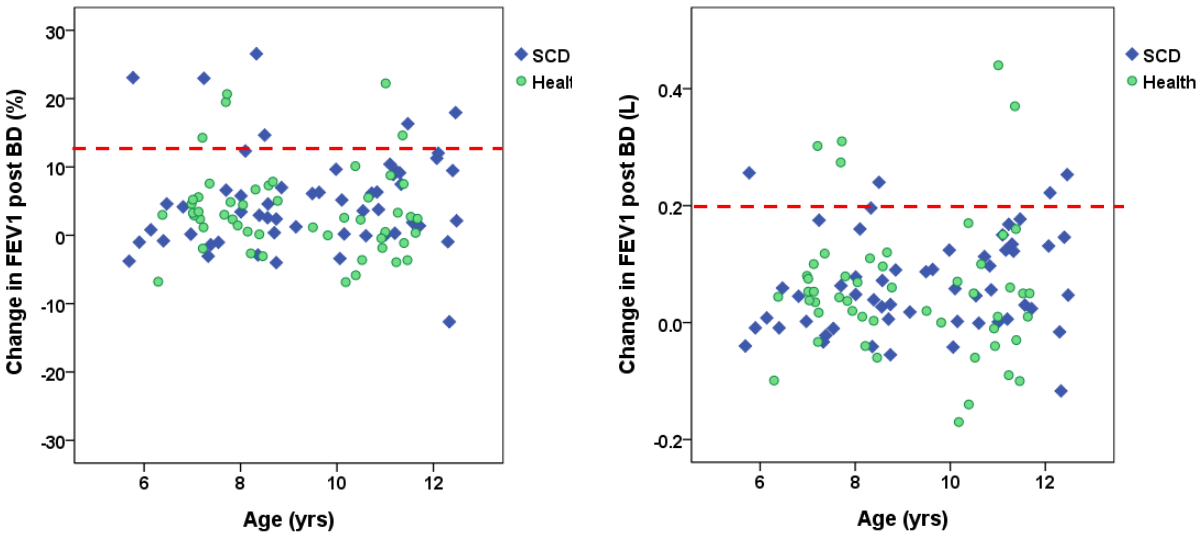

Figure 7-5: Relationship between age and change in FEV_1 post bronchodilator in 50 healthy children and 55 children with SCD

Results presented as absolute (L) or percent change. Red dashed line indicates the published threshold for reversibility (12% or 200 mL).

There was no significant relationship between age and absolute change post BD (r=0.07) or percent change post BD (r=0.01).

The mean change post bronchodilator +/-1.96 SD seen in health was used to define the thresholds of reversibility, and demonstrated no significant BDR in the group of children with SCD (Figure 7-6).

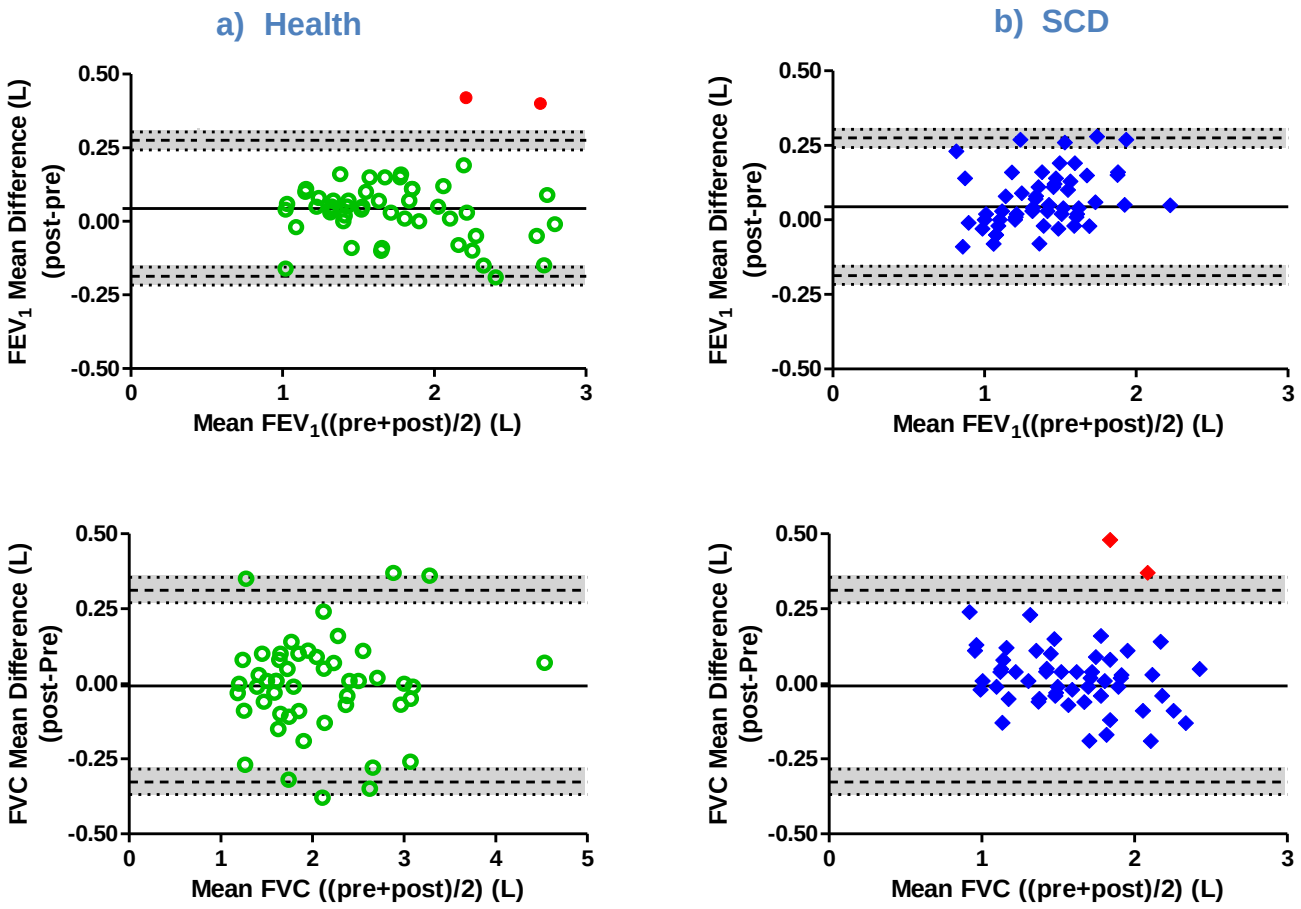

Figure 7-6: BDR in health and SCD for FEV_1 and FVC.
Legend: Dashed lines denote 95% limits of agreement calculated from the pre and post bronchodilator values obtained in health. The grey shaded area denotes the 95% CI around these limits. Red dots indicate those children in whom a significant BDR was observed (i.e. change outside the limits agreement determined in health).

7.8 Summary

The ethnic-specific spirometry equations by Wang *et al*,[5] alongside a healthy, ethnically matched control group provided adequate reference data with which to interpret spirometry data obtained in children with SCD. There was a clinically significant reduction in FEV_1 and FVC in children with SCD in comparison to healthy children; however they were reduced in equal proportions, such that there was no difference in the FEV_1/FVC ratio. Assessments of bronchodilator responsiveness revealed that children with SCD had no increased BDR compared with that seen in health. Baseline spirometry results and the lack of BDR suggest either the presence of restrictive lung disease or physiologically reduced lung capacity secondary to poor growth in children with SCD. These results are evaluated alongside the other lung function measurements reviewed previously and the clinical symptoms in the next chapter.

8 Correlation of lung function tests with respiratory morbidity in children with SCD

8.1 Introduction

The previous four results chapters in this thesis have reviewed the methodology and interpretation of four commercially available lung function tests (Impulse Oscillometry (IOS), specific airways resistance (sR_{aw}), plethysmographic lung volumes and spirometry) in children aged four to twelve years. After developing recommendations for the applications and interpretation for each test, each individual test was applied in children with SCD. This final results chapter summarises the results obtained so far and compares the various lung function outcomes from these tests to one another and in relation to the clinical symptoms.

8.2 Aim

The primary aim of this chapter was to ascertain the most appropriate combination of lung function tests for the clinical monitoring of children with SCD with suspected lung disease.

8.3 Objectives

The primary objectives were

i. To compare results obtained from each lung function test and delineate correlations between the measurements
ii. To evaluate the clinical symptoms in combination with the lung function results
iii. To determine the most appropriate combination of lung function outcomes to detect lung disease in children with SCD

8.4 Hypothesis

All lung function techniques of interest in this thesis will detect differences between healthy children and children with SCD with pulmonary manifestations.

8.5 Subjects with matched lung function assessments

Due to the nature of the protocol and the age limits involved for each assessment, not all children underwent the entire protocol. To describe the most appropriate combination of lung function measurements for monitoring SCD and the relationship between various outcomes, matched measurements conducted in the same children were evaluated.

Fifty-nine children with SCD had matched measurements of IOS, sR_{aw} and spirometry measurements on the same occasion, and 53 children had matched spirometry and lung volume measurements (only 20 of these children had sR_{aw} measurements). There were no significant differences between the demographics for each of these subgroups either when compared with each other or to the main study group; however the children who underwent plethysmographic lung volume measurements were slightly older than those in whom matched IOS, sR_{aw} and spirometry results were obtained (Table 8-1).

Table 8-1: Demographics of the subsets of children with SCD in whom matched lung function measurements on the same test occasion were obtained.

	Matched data for IOS, sRaw and spirometry	Matched data for Lung volumes and spirometry
n (% male)	59 (54%)	53 (43%)
Age (yrs)	7.5 (2.1)	9.8 (1.8)
Height Z	0.1 (1.1)	0.0 (1.1)
Weight Z	0.0 (1.0)	-0.1 (1.1)
BMI Z	0.0 (1.0)	-0.1 (1.1)

Demographics of entire population studied can be found in Table 3-2
Height, weight and BMI were expressed as Z Scores according to the CDC growth charts[131]

8.6 Summary of previous results chapters

Each lung function test was reviewed independently in the relevant chapter. The principle findings from each chapter were:

- Significant differences in IOS reference data based on White children[41] and healthy Black children occurred
  - R_{15} and R_{20} were *lower* in the presence of SCD compared with healthy Black children
- No significant differences in sR_{aw} outcomes occurred between healthy Black and White children, nor between healthy Black children and children with SCD
- Significant ethnic differences in lung volumes occurred however the magnitude and direction of the differences were dependent on outcome:
  - FRC and TLC were 0.7 Z Scores (14%) and 0.5 Z Scores (6%) lower in Black children than White children
  - RV/TLC was 0.5 Z Scores (10%) higher in Black children
    - RV was 0.1 Z Scores (4%) higher in Black children (not significant).
- Interpretative strategies (ethnic adjustments) recommended by the ATS/ERS[2] were not appropriate for interpreting lung volumes in Black children.
- When compared with the healthy Black children of the same age, the children with SCD were found to have a significantly reduced TLC (-0.8 Z Scores) and borderline significantly elevated RV/TLC (0.3 Z Scores higher than the controls).
- Ethnic-specific spirometry equations by Wang[5] were suitable for interpreting spirometry in Black and White children
- There was a clinically significant reduction in FEV_1 and FVC in children with SCD in comparison to healthy Black children
- BDR observed in SCD was similar to that seen in health, and was therefore clinically insignificant.

The following section summarises the main conclusions for each lung function test and the main findings are collated in Table 8-2, whilst the retrospective power calculations based on the actual number of children assessed can be seen in Table 8-3.

8.6.1 Summary of IOS results

In chapter 4, two paediatric IOS reference equations based on White children[41,65] were evaluated and found to differ from one another in such a way that they were not interchangeable. Furthermore, marked differences between predicted values and those measured were observed: Healthy Black children had, on average, resistance and reactance measurements that were ~0.6 Z Scores higher and 0.8 Z Scores lower than predicted (based on White children) which could potentially be due to ethnic differences. The most appropriate method of interpreting IOS data in Black children was found to be direct comparisons between health and disease using Z Scores derived from Dencker equations,[41] although true limits of normality could not be defined. Multiple regression analyses and direct comparisons demonstrated that, after adjusting for age and height, having SCD did not have a significant impact on the R_5 and R_{10} values; however R_{15} and R_{20} were *lower* in the presence of SCD. X_5 was also significantly more negative in the presence of SCD after adjusting for height, whilst the outcomes affected most by SCD were AX and $Fdr_{5\text{-}15}$, which were both significantly elevated (commonly associated with peripheral airway obstruction).

There were no significant differences between the BDR in health and SCD when R_5 and R_{10} were compared, however there was a significant *increase* in R_{15} post BD in some children with SCD thus contributing to the differences seen in $Fdr_{5\text{-}15,}$ which identified five children with SCD with a significant BDR (over and above that seen in health). AX identified just one child with SCD with a clinically significant BDR.

8.6.2 Summary of sR_{aw} results

Chapter 5 (sR_{aw}) comprised the largest collation of paediatric sR_{aw} data from healthy controls to date. A comprehensive review of the different methodologies across the five collaborating centres was undertaken, and results from the study enabled the development of a quality-control over-read sheet and recommendations for future measurements. Furthermore, preliminary sex-specific reference equations, which adjusted for the minimal age-related changes in sR_{eff} and sR_{tot}, were developed.[128] These reference equations, although developed for White children, were shown to be applicable in Black children, since no ethnic differences were observed. Other than a slight trend towards children with SCD adopting a lighter breathing pattern, there were no significant differences in baseline sR_{aw} outcomes or response to bronchodilator between SCD and healthy children.

8.6.3 Summary of Plethysmographic Lung Volume results

Chapter 6 consisted of an assessment of plethysmographic lung volumes in children to assess the extent to which pre-defined QC criteria were applicable and the impact of ethnicity. Published QC recommendations appeared to be suitable for children undergoing these measurements, and within-test repeatability was similar to that previously reported.[94,149] Two reference equations based on White children were reviewed,[97,98] and ethnic differences were observed, however the magnitude and direction of the differences were dependent on the lung volume outcome and the reference equation selected. Predicted values described by Rosenthal *et al*[98] appeared to be the more reliable method of interpretation; however they could only be used in Black children if suitable adjustments both to the predicted values and limits of normality were implemented. Finally plethysmographic lung volumes were measured in children with SCD and results compared to those from healthy Black children of similar age (6 to 12 years). With the exception of TLC, which was significantly reduced in the SCD group (indicating restrictive lung disease), there were no statistical group differences in lung volume outcomes between children with SCD and healthy Black children. Lung volumes were not used as an outcome measure for bronchodilator responsiveness.

8.6.4 Summary of spirometry results

The spirometry results chapter (chapter 7) reviewed the most comprehensive spirometric reference data to date (the "All-Age" equations by Stanojevic *et al*,[126]) along with the Black specific reference data by Wang *et al*.[5] As expected ethnic differences in spirometry were observed, and the ethnic-specific spirometry equations by Wang,[5] alongside a healthy, ethnically matched control group proved to be the most feasible method of interpreting spirometry data from Black children at the time of writing this thesis. A comparison of children with SCD, to healthy Black children of the same age demonstrated a clinically significant reduction in FEV_1 and FVC in children with SCD in comparison to healthy children; however these outcomes were reduced in equal proportions, such that there was no difference in the FEV_1/FVC ratio. In addition, assessments of bronchodilator responsiveness revealed that children with SCD exhibited no increased BDR compared with that seen in health when assessed using spirometry.

Table 8-2: Summary of the lung function tests undertaken in healthy Black children and children with SCD.

Test	Children assessed:			Lung function outcome	Mean Diff (SCD-health) (95% CI)	BDR: change from baseline		
	SCD (n)	Control (n)	Age (yrs)			SCD	Control	Mean Diff (95%CI) (SCD – health)
IOS	59	68	4-11	R_5 Z Score[a]	**0.4 (0.0 ; 0.7)***	-0.9 (0.8) Z	-1.1 (1.2) Z	0.1 (-0.3; 0.6)
				R_{10} Z Score[a]	-0.2 (-0.4 ; 0.1)	-0.8 (0.9) Z	-1.1 (1.0) Z	0.3 (-0.2; 0.7)
				R_{15} Z Score[a]	**-0.6 (-0.8 ; -0.3)*****	0.3 (1.1) Z	-0.7 (1.1) Z	**1.0 (0.4; 1.5)*****
				AX ($kPa{\cdot}L^{-1}$)	**1.5 (1.0; 2.0)*****	-1.1 (1.0) $kPa{\cdot}L^{-1}$	-1.0 (1.4) $kPa{\cdot}L^{-1}$	-0.1 (-0.6; 0.5)
				Fdr_{5-15} ($kPa{\cdot}L^{-1}.s$)	**0.2 (0.1; 0.2)*****	-0.2 (0.1) $kPa{\cdot}L^{-1}.s$	-0.1(0.1) $kPa{\cdot}L^{-1}.s$	**-0.1 (-0.1; -0.00)*****
sRaw	99	56	4-10	sR_{tot} Z Score[b]	-0.2 (-0.5; 0.1)	-0.7 (0.9) Z	-1.2 (0.7)Z	0.5 (0.1; 1.0)
				sR_{eff} Z Score[b]	-0.3 (-0.6; 0.1)	-0.8 (0.8) Z	-1.3 (0.7)Z	0.5 (0.1; 0.8)
Lung Volumes	85	68	6-12	FRC *adjusted* Z[c]	0.0 (-0.3; 0.3)	Not applicable		
				RV *adjusted* Z[c]	-0.2 (-0.4; 0.1)			
				TLC *adjusted* Z[c]	**-0.8 (-1.1; -0.5)*****			
				RV/TLC *adjusted* Z[c]	0.3; (0.0; 0.6)[†]			
Spirometry	60	214	6-12	FEV_1 % pred.[d]	**-12.0 (-15.6; -8.4)*****	5.0 (7.3)%	3.1 (6.0) %	1.9 (-0.7; 4.5)
				FVC % pred.[d]	**-13.8 (-17.6; -10.0)*****	2.6 (8.3)%	0.5 (7.8) %	2.11 (-1.0; 5.2)
				FEF_{25-75} % pred.[d]	**-12.8 (-21.6; 4.1)*****	19.4 (31.4)%	19.1 (23.9) %	0.3 (-10.6; 11.3)
				FEV_1/FVC % pred.[d]	1.6 (-0.6; 3.8)	2.7 (6.6) %	3.4 (7.7) %	-0.7 (-3.5; 2.1)

[†]p=0.52, *p<0.05, **p<0.01, ***p<0.001 Results in bold also indicate significance.
Where applicable, published reference equations were applied to each outcome: IOS[a]=Dencker[41]; sR_{aw}[b]=Kirkby[128]; Lung volumes[c]=Rosenthal (with ethnic adjustment described in chapter 5)[98] and spirometry[d] = Wang[5]

Table 8-3: Retrospective sample size calculation on baseline measurements obtained in healthy children and children with SCD

	Actual sample size:		Retrospective power calculation:		
	SCD	Control	Power	Significance	Difference in SD (absolute values)
IOS	59	68	80%	0.05	0.52 SD (0.05 $kPa \cdot L^{-1}.s$ in Fdr_{5-15} or 0.52 $kPa \cdot L^{-1}$ in AX)
sR_{aw}	99	56	80%	0.05	0.53 SD (0.1$kPa \cdot s$ in sR_{eff})
Lung Volumes	85	68	80%	0.05	0.48 SD (6%pred in FRC; 9.6% pred in RV; 4.7% pred. in TLC; or 9.1% pred. RV/TLC)
Spirometry	60	214	80%	0.05	0.2 SD (or 2.4% predicted).

1 SD is equivilent to 1 Z Score. Therefore with these numbers there is sufficient power to detect a difference of 0.2 Z Scores in spirometric outcomes, but only ~0.5 Z Scores for IOS and plethysmography outcomes.

8.7 Symptoms and Doctor Diagnosis of Asthma in children with SCD

In addition to the combination of lung function measurements undertaken, respiratory symptoms were documented. The following questions from the health questionnaire (see appendix) were analysed to determine respiratory symptoms/ history of asthma:

1. Does the child cough on most days (>4 days per week)?
2. Does the child wheeze on most days (>4 days per week)?
3. Has a doctor ever said the child has asthma?
4. Does the child take asthma medication?

All of the healthy children included in the study were free from respiratory symptoms, and none had a doctor diagnosis of asthma or received any medication for asthma (i.e. they answered "no" to all the above questions) (Table 8-4).

Table 8-4: Frequency of children with a positive response to the respiratory health questionnaire

	Cough most days	Wheeze most days	Asthma diagnosis	Asthma medication
Health (n=68)	0%	0%	0%	0%
SCD (n=59)	50%	14%	12%	14%

Half of the 59 children with SCD, in whom three lung function measurements were obtained, reported cough on most days. Eight children (14%) reported wheeze most days, seven (12%) had a doctor diagnosis of asthma and a further eight (14%) were receiving asthma medication at the time of assessment (Table 8-4). In total 10 (17%) of the children with SCD had answered yes to at least one of the questions: wheeze most days, doctor diagnosis of asthma and/or asthma medication. "Cough most days" was excluded from the "respiratory symptom" coding criteria, as it was impossible to identify differences with such a large proportion complaining of cough. Of the ten children reporting respiratory symptoms other than cough, seven had been referred to a specialist respiratory physician, and a further eight children who did not complain of respiratory symptoms were reviewed in a respiratory clinic, regardless of symptoms. Thus 25% of the children with SCD reviewed in this study were receiving specialist follow-up with a respiratory physician. In addition, 30% of the children with SCD had 1 or 2 hospital admissions due to acute chest syndrome (ACS), and a further 13% had 3

or more episodes of ACS, however at the time of writing this thesis the ACS information could not be paired with the lung function data.

In light of the respiratory symptoms/respiratory follow-up, the various lung function outcomes were reviewed in combination with one another to elucidate the nature of the underlying pathophysiology of SCD, to determine concordance and to identify which lung function outcomes detected the most abnormalities. Table 8-5 shows the correlation of each individual lung function outcome against one another and respiratory symptoms.

IOS and sR_{aw} outcomes demonstrated significant but weak correlations (r =0.36 and 0.35 for sR_{eff} Vs. AX and sR_{eff} Vs. Fdr, respectively), whereas the strongest correlations occurred between spirometry and lung volume outcomes (Table 8-5). The various combination of assessments, along with cross comparisons are investigated further in the following sections.

Table 8-5: Pearsons correlation of differing lung function outcomes and respiratory symptoms in children with SCD aged 4 to 12 years.

	AX kPa·L^{-1}	Fdr kPa·L^{-1}.s	sReff Z Score	FEV_1 %Pred	FVC %Pred	FEV_1/FVC %Pred	FRC Z Score	RV Z Score	TLC Z Score	RV/TLC Z Score	Resp. symptoms
AX(kPa·L^{-1})		**0.89*** **	**0.36** **	-0.20	-0.21	0.20	*0.08*	*0.16*	*0.01*	*-0.18*	0.21
Fdr (kPa·L^{-1}.s)			**0.35** **	-0.20	-0.22	0.18	*-0.16*	*-0.33*	*-0.15*	*-0.3*	0.19
sR_{eff} Z Score				0.16	0.24	-0.03	***0.58** *	*0.22*	***0.6** **	*-0.12*	-0.17
FEV_1 %Pred					**0.92*** **	0.08	**0.56** *	0.02	**0.77*** **	**-0.54** **	0.19
FVC %Pred						-0.02	**0.71** **	0.08	**0.86*** **	**-0.55** *	**-0.44** **
FEV_1/FVC %Pred							0.0	-0.08	-0.02	-0.11	0.16
FRC Z Score								0.13	**0.67** **	-0.32	-0.26
RV Z Score									**0.49** **	**0.66** **	0.09
TLC Z Score										-0.23	-0.28
RV/TLC Z Score											0.18
Resp. symptoms											

*p<0.05; **p<0.01; ***p<0.001.

Footnote: Lung function outcomes were compared in children with matched results. 59 children had matched IOS, sR_{aw} and spirometry results and 53 children had matched spirometry and lung volume results. Since there were only 19 matched datasets of lung volumes and IOS and lung volume and sR_{aw}, these results are highlighted in blue italics as sample size prevents a conclusion on these results.

8.8 Combination of lung function assessments

Chapter 4 investigated the best methods of interpreting IOS results and found severe limitations in the reference data. Consequently direct comparisons of AX and Fdr_{5-20} were made as these outcomes take into account changes in reactance and resistance across a range of frequencies. Limits of normality for AX and Fdr_{5-20} however, have yet to be established. Furthermore the conventional methods for calculating limits of normality (mean +/-1.96 SD) were inappropriate since AX and Fdr_{5-20} were not normally distributed. The two outcomes were therefore plotted against one another and an upper limit was estimated by drawing a line across the plot where the majority of healthy subjects fell below these lines. A crude estimate for an upper limit of normality was 0.45 $kPa \cdot L^{-1}.s$ for Fdr and 5.0$kPa \cdot L^{-1}$ for AX (Figure 8-1).

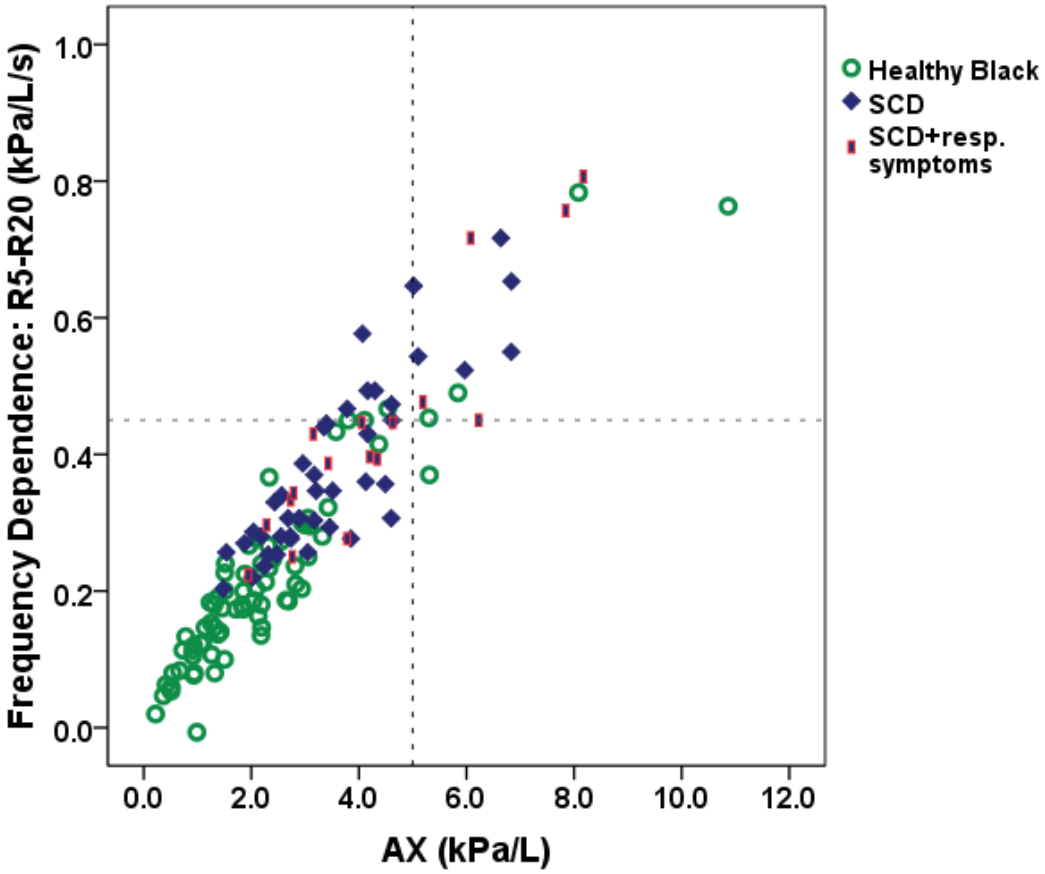

Figure 8-1: Comparison of IOS outcomes in 68 healthy Black children and 59 children with SCD.
Legend: Estimates of the upper limit of normality were made by adding a reference line for each outcome denoting the point whereby 95% of healthy children fell below that value. Upper limit for Fdr_{5-20} was estimated as 0.45$kPa \cdot L^{-1}.s$ and AX was estimated as 5.0$kPa \cdot L^{-1}$.

Using the estimated upper limits defined in Figure 8-1, IOS outcomes could be compared with spirometry (Figure 8-2) and sR_{aw} outcomes (Figure 8-3).

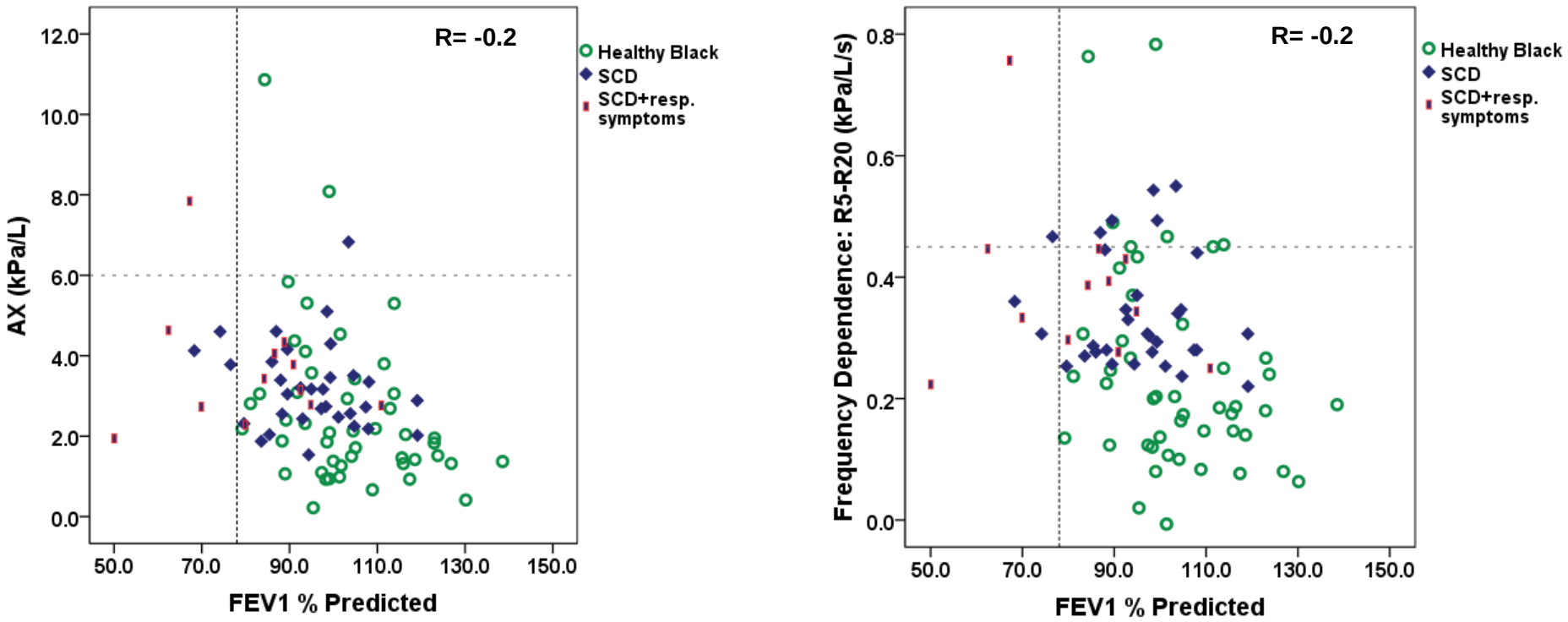

Figure 8-2: Comparison of FEV_1 % predicted and IOS outcomes (AX and Fdr_{5-20}) in 59 children with SCD and 68 healthy Black children aged 4-11y.
Legend: Vertical black dotted line indicates the lower limit of normal for FEV_1 % predicted, and the horizontal grey dashed line indicates a visual estimate of the upper limit of normal for AX (where the majority of healthy children fall below this line). Results in the upper left quadrant are abnormal for both measurements
AX results in SCD and health significantly overlap, and there was no correlation between FEV_1 and AX (p=0.15, r=-0.2). AX is not useful for establishing abnormality in individual cases.

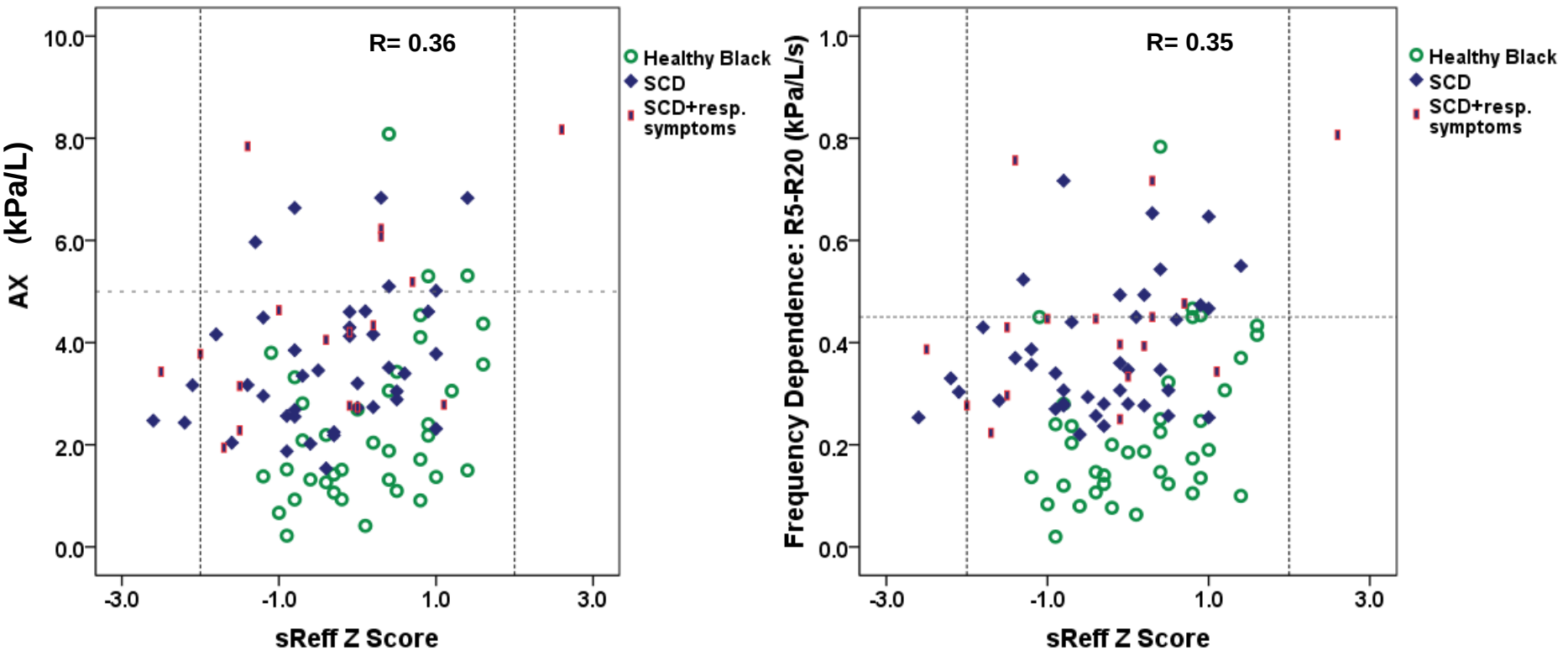

Figure 8-3: Comparison of sR_{eff} Z Score and IOS (AX and Fdr_{5-20}) in 59 children with SCD and 68 healthy Black children aged 4-11y.
Legend: Vertical black dotted line indicates the upper and lower limit of normal for sR_{eff} Z Score (+/-1.96SD), and the horizontal grey dashed line indicates a visual estimate of the upper limit of normal for AX (where the majority of healthy children fall below this line). Results in the upper right quadrant are abnormal (increased resistance) for both measurements, whereas results in the lower left quadrant are consistent with reduced airways resistance.
Weak correlations between IOS outcomes and sR_{eff} were observed. The majority of children with SCD had sR_{eff} results within the normal limits, with a few children presenting with unexpectantedly reduced sR_{eff} and concordant lower AX and Fdr.

A comparison of IOS outcomes (AX and Fdr) and FEV_1 revealed a trend toward those children with lower FEV_1 % predicted also having higher AX or Fdr values, thus supporting the evidence of mild airway obstruction in these children (Figure 8-2). Similarly there was a tendency for those children with respiratory symptoms to have higher AX and Fdr and lower FEV_1, although this did not reach statistical significance. Despite these trends, IOS outcomes failed to provide any additional information regarding possible pathology other than already identified by spirometry and the respiratory health questionnaire.

Figure 8-3 plots IOS outcomes against sR_{eff}. There was a weak correlation between these outcomes with concordant results between low sR_{eff} and low AX and Fdr, and one child with concordant elevated sR_{eff} Z Score and elevated AX and Fdr (i.e. this child's results fell in the top right quadrant for both plots). This child had reported significant respiratory symptoms, significant bronchodilator responsiveness and was undergoing respiratory follow-up with the specialist respiratory physician (i.e. results consistent with obstructive lung disease). The associated reduced IOS outcomes and sR_{eff} may be suggestive of reduced resistance in the central airways (noted by the decreased resistance at high frequencies (R_{20}) measured in IOS (chapter 4)) which may be the consequence of restrictive lung disease (discussed further in section 9.4.6)

Further comparisons of sR_{eff} and spirometry (FEV_1) were made (Figure 8-4). No significant differences in sR_{eff} between health and SCD where observed, and the majority of sR_{eff} results fell within the normal limits. Five children (8%) with SCD fell below the lower limits of normal for sR_{eff}. Two of these children reported respiratory symptoms, but neither were being reviewed in the respiratory clinic. On comparison to FEV_1 % predicted, the few children with low sR_{eff} did not have any evidence of increased expiratory airflows (Figure 8-4) and a similar pattern was seen in other spirometry outcomes (Table 8-5). Contrary to the expectation that an elevated sR_{aw} may be associated with reduced flows, there was no correlation between sR_{eff} Z Score and FEV_1 % predicted ($p=0.3$, $r = 1.55$), or sR_{eff}. In summary, more abnormalities (i.e. results falling outside the limits of normality) were detected by spirometry than sR_{eff}.

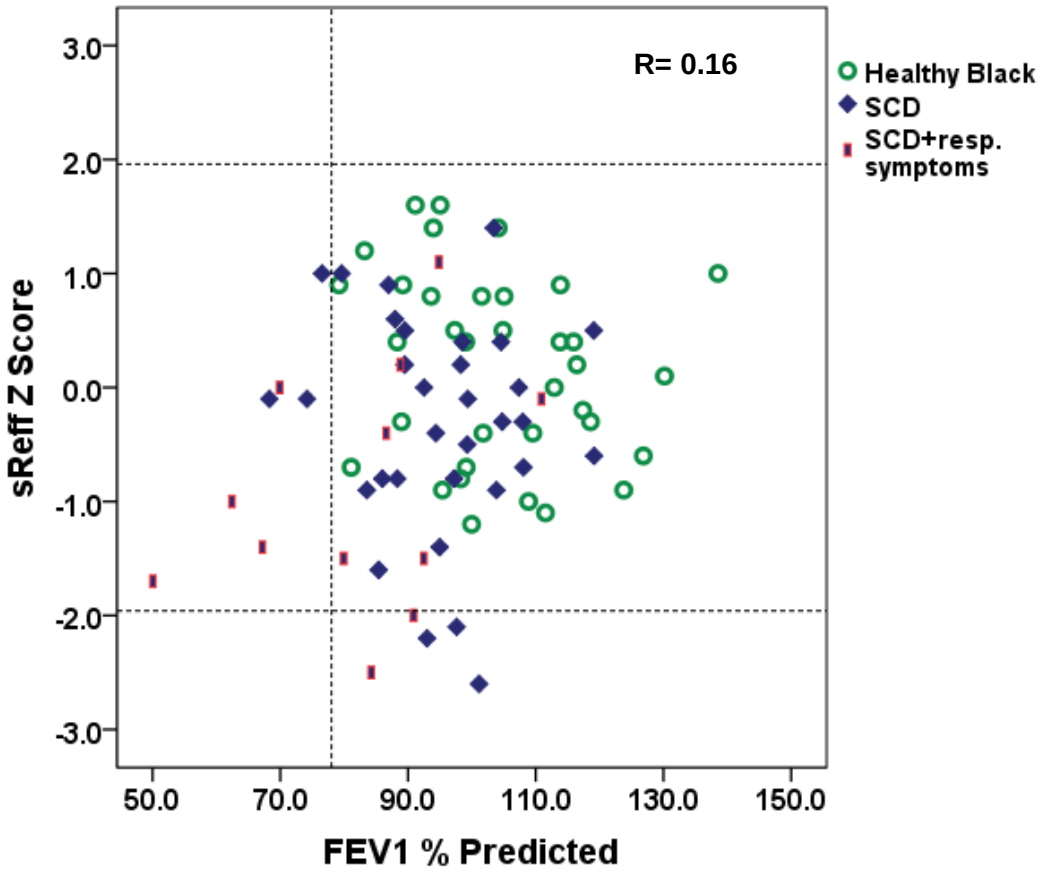

Figure 8-4: Comparison of sR_{eff} Z Score and FEV_1 % predicted in 59 children with SCD and 68 healthy Black children aged 4-11y.
Legend: Horizontal dotted line indicates the upper and lower limit of normal for sR_{eff} Z Score (+/-1.96SD), and vertical dotted line indicates the lower limit of normal for FEV_1 % predicted. There was no correlation between FEV_1 % predicted and sR_{eff} Z Score. More children with abnormal results were identified by FEV_1 than by sR_{eff}.

Matched sR_{eff} and lung volume results were available in 20 children with SCD (the children from St Louis did not undergo sR_{aw} measurements, and initial lung volume measurements in London were not accompanied by sR_{aw} measurements). Despite the limited numbers a significant correlation between FRC and sR_{eff} (r=0.58) and TLC and sR_{eff} (r=0.6) was observed. No child with SCD had elevated sR_{eff}, whereas five children, including two symptomatic children had low sR_{eff}, however these were not the same children in whom FEV_1 was reduced (suggesting restriction). Those with the lowest TLC Z Score also had the lowest sR_{eff} Z Score, suggesting low sR_{aw} measurements are associated with restrictive lung disease, potentially due to the low FRC values observed in restrictive lung disease. (Figure 8-5).

Further evidence of restriction was shown in Figure 8-6 which demonstrated highly significant correlations between spirometry and lung volume outcomes.

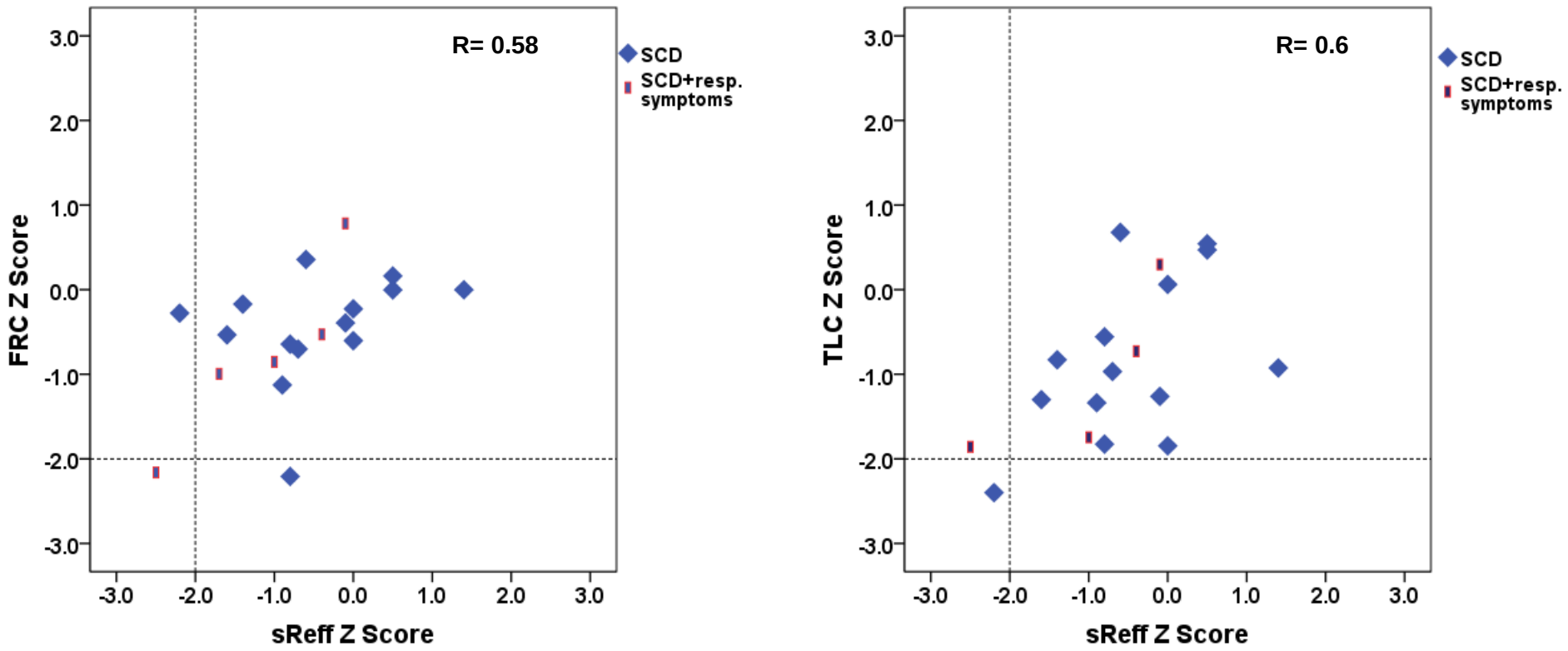

Figure 8-5: Comparison of sR_{eff} Z Score and Lung volumes (FRC and TLC Z Scores) in 20 children with SCD aged 6-12y
Legend: Dotted line indicates the lower limit of normal
The two outcomes are significantly correlated; however there was no relationship between those reporting respiratory symptoms and the lung function results.
NB: Interpretation is limited due to the relatively small sample of children in whom concordant measures were obtained.

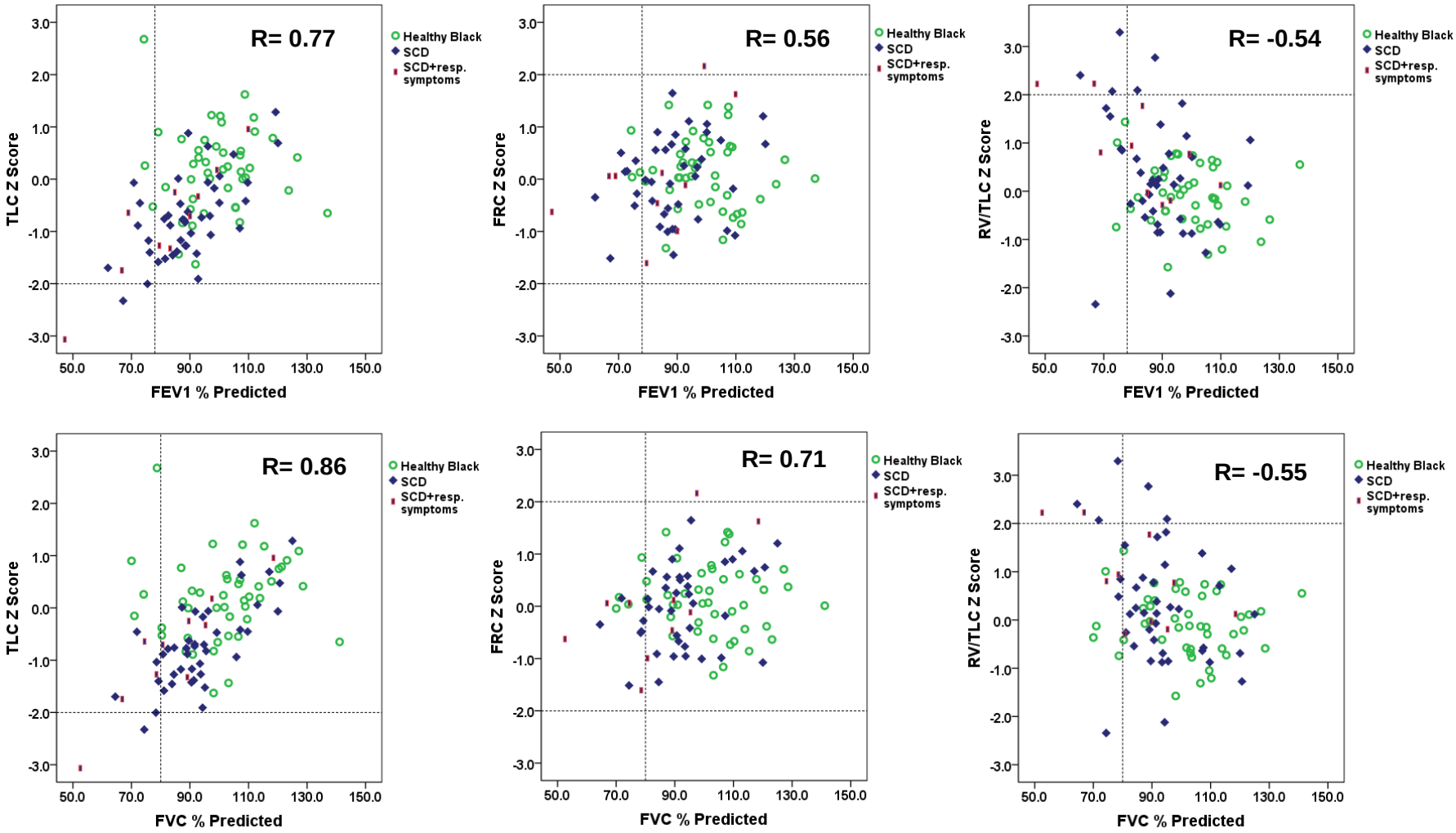

Figure 8-6: Comparison of FEV_1 and FVC %pred and FRC, TLC and RV/TLC Z Scores in 53 children with SCD and 68 healthy Black children.
Legend: Dotted line indicates the lower limit of normal. Significant correlations were seen in all outcomes

Spirometry and lung volume outcomes were compared. No significant correlation between RV and any spirometric outcomes were observed (Table 8-5), hence these results were not plotted. Highly significant correlations between FEV_1 % predicted and TLC Z Score ($r=0.77$, $p<0.0001$) and FRC ($r=0.56$, $p<0.05$) were observed with an even greater correlation when comparing FVC % predicted with these two outcomes ($r=0.86$ and 0.71 for TLC and FRC respectively) ($p<0.01$). In addition, a significant correlation between FEV_1 and FVC % predicted and RV/TLC was observed ($r=-0.54$ and -0.55 respectively) (Table 8-5 and Figure 8-6). Those children with low FEV_1 and FVC % predicted had concurrent reduced lung volumes (TLC and FRC) indicative of restriction, whereas those children with an elevated RV/TLC also had reduced FEV_1 and FVC % predicted.

8.9 Summary

In summary, four commercially available lung function tests were evaluated in healthy children aged 4 to 12 years and then applied in children with SCD to determine the extent to which these children had lung disease/abnormalities in lung function. A respiratory health questionnaire was administered and all lung function outcomes were reviewed in light of the clinical symptoms. Fifty percent of the children with SCD reported cough on most days, whilst 25% had been reviewed by a specialist respiratory consultant in the three months previous to the assessments. Despite the relatively high proportion of respiratory symptoms reported, the proportion of children with lung function results falling outside the limits of normal was relatively small: FEV_1 % predicted in children with SCD was significantly reduced compared to that in control children, with 15% of children with SCD assessed falling below the limits of normality. FVC however, was reduced to a similar extent, indicative of a restrictive rather than obstructive pattern. Concurrently, a group reduction in TLC was observed in children with SCD, and a comparison of the two lung function tests revealed those children with low TLCs also had reduced FEV_1 and FVC % predicted. Around 20% of children with SCD were identified as having restrictive lung disease (defined by a reduced TLC).[94] Although lung volumes are the gold standard for identifying restrictive lung disease[94] the measurement is technically more difficult to perform, and every child with a reduced TLC had concurrently low FEV_1 and FVC % predicted. Thus, in this study, the use of plethysmographic lung volumes helped to confirm diagnosis, rather than add further information to the clinical picture.

Whilst no group differences in sR_{aw} were detected between healthy children and children with SCD, there was a trend for a slightly reduced sR_{aw} in SCD which correlate

with the low lung volumes observed in these children. However, measurements of sR_{aw} were not found to discriminate between healthy children and those with SCD. Similarly, measurements of IOS were not a useful adjunct to the lung function testing, as interpretation was limited due to the lack of well-defined limits of normality. Despite a rough estimate of the upper limit of normal (based on the healthy children) and significant group differences between health and SCD, IOS outcomes did not yield any additional clinical information, and results did not correlate with clinical symptoms.

In summary, the combination of four lung function measurements, a respiratory questionnaire, and a healthy control group of children of the same age and ethnicity revealed children with SCD to have reduced TLC, with concurrent reductions in FEV_1 and FVC. No differences in sR_{aw} were observed and IOS outcomes proved to be of limited value due to poorly defined limits of normality, and the huge scatter around the mean. The highest proportion of abnormal results was detected by spirometry, and the only outcome that was significantly associated with respiratory symptoms was FVC. Children with SCD did not demonstrate clinically significant bronchodilator responsiveness regardless of the outcome measured. The results from this study suggest a pattern of restrictive lung disease in children with SCD. Of the outcomes assessed in this thesis, spirometry appears to be the most useful outcome measure for routine assessment of lung disease in SCD.

9 Discussion

9.1 Introduction

The overall aims of this thesis were to improve the application and interpretation of lung function measurements in young children and to determine the extent to which ethnic differences in lung function occurred between healthy Black and healthy White children after adjusting for height, sex and age. A series of investigations of four commercially available lung function tests (Impulse oscillometry (IOS), specific airways resistance (sR_{aw}), plethysmographic lung volumes, and spirometry) involving a total of 214 healthy Black and 186 healthy White children aged 4 to 12 years were undertaken. In addition, 2,872 sets of sR_{aw} data from 2,347 children were collated as part of the Asthma UK Initiative to develop sR_{aw} reference equations.

As a result of the study, recommendations for the application (in terms of data collection and measurement conditions) and interpretation (in terms of appropriate reference data, limits of normality and ethnic adjustments) for each lung function test were made. The lung function tests were then applied in a group of up to 85 children with Sickle Cell Disease (SCD) to determine the extent to which each outcome measure identified lung function abnormalities in these children. Each lung function test was reviewed in isolation, and then in combination with the other lung function assessments and the clinical status of the child. This final discussion chapter will summarise the principal findings of the thesis, identify the strengths and weaknesses of the study, compare findings with published literature, highlight the implications of the results and explore areas for future research.

9.2 Principal findings reported in this thesis

Chapter 8 summarised the principal findings of the thesis with regards to development of recommendations for the application and interpretation of four commonly used lung function tests and the application of these measurements in children with SCD, both as stand-alone assessments and in combination with other lung function tests and clinical symptoms derived from a questionnaire.

9.2.1 Novel findings

This thesis comprised the largest collation of paediatric sR_{aw} data from healthy controls to date, and developed preliminary sex-specific reference equations which adjusted for the minimal age-related changes in sR_{eff} and sR_{tot}, along with recommendations for their use.[128] Additional novel findings of the thesis were:

- Resistance within the central airways was lower (significant reduction in R_{15} and R_{20}) in children with SCD compared with healthy Black children.
- Considerable misinterpretation of plethysmographic lung volume data from Black children would occur if current paediatric reference data and recommended ethnic adjustments were applied. New ethnic adjustments for interpreting lung volumes in Black children were developed[146]
- Spirometry and plethysmographic lung volumes were the most useful lung function assessments for monitoring children with SCD.

9.2.2 Comparison of results to main hypotheses

9.2.2.1 Chapter 4 hypothesis

IOS data from healthy Black children will be significantly different to that predicted by reference data derived from White children

Discrepancies between IOS predicted values based on White children[41] and healthy Black children were observed: resistance at all frequencies were ~0.5 Z Score higher, and X_5 was ~0.8 Z Score lower in Black children.

9.2.2.2 Chapter 5 hypothesis

There are no ethnic differences in sR_{aw} between Black and White children.

No significant differences were observed between sR_{aw} Z Scores[128] obtained from healthy Black and White young children.

9.2.2.3 Chapter 6 hypothesis

Lung volume data from healthy Black children will be significantly lower than that predicted by reference data derived from White children.

Significant ethnic differences in lung volumes occurred however the magnitude and direction of the differences were dependent on outcome, such that considerable misinterpretation of lung disease would have occurred had interpretation been based on White reference data[98] or using the simple ethnic adjustments recommended by the ATS/ERS.[2] There were no significant differences in RV between healthy Black and White children, however, TLC was found to be ~8% lower in Black children. A 12% adjustment in these outcomes would have over-estimated the predicted values for RV

and TLC in Black children and potentially over-diagnosed obstructive lung disease (indicated by a raised RV) and under-diagnosed restrictive lung disease (defined by a reduced TLC). Furthermore, despite the difference in FRC between Black and White children being similar to that indicated by the ATS/ERS[2] (~6% lower in Black children), predicted values for FRC based on Rosenthal equations[98] were found to be over-estimated for White children, thus the extent of hyperinflation (identified by an elevated FRC) would be potentially misdiagnosed in these children, furthermore the degree of restrictive lung disease may be under-estimated.

9.2.2.4 Chapter 7 hypothesis

Healthy Black children will generate different predicted values and lower limits of normal for spirometry in comparison to those defined by previously published reference data based on White children.[126]

The All-Age[126] spirometry equations demonstrated excellent agreement with White children, but were not appropriate for Black children unless ethnic adjustments were applied. Wang *et al* ethnic specific spirometry equations[5] were shown to be adequate for interpreting spirometry in children aged 6 to 12 years.

9.2.2.5 Chapter 8 hypothesis

All lung function techniques of interest in this thesis will detect differences between healthy children and children with Sickle Cell Disease with pulmonary manifestations.

There were no significant group differences in sR_{aw}, although there was a tendency for sR_{aw} to be *lower* in SCD compared to health. Furthermore, there was no significant difference in resistance measured at low frequencies by IOS (reflecting more peripheral airway calibre), whilst IOS resistance at high frequencies, which tends to reflect more central airway calibre, were significantly *lower* than that seen in health, resulting in increased frequency dependence of resistance (Fdr) in those with SCD. This Fdr was not, however, due to the normal pattern of increased peripheral airway resistance more commonly seen in obstructive lung disease.

Spirometric and plethysmographic lung volume results revealed significant group differences between children with SCD and healthy Black children, with FEV_1, FVC and TLC being significantly lower in the children with SCD thus indicating a pattern of restrictive lung disease. Despite a relatively high proportion of respiratory symptoms reported in SCD, few were on anti-asthma medication and BDR was not detected, nor was there evidence of airway obstruction or increased airways resistance. Results suggested a pattern of restrictive lung disease in SCD and that spirometry and plethysmographic lung volumes were the most useful lung function tests in assessing children with SCD.

9.3 Strengths and weaknesses

The strengths of the study include the large number of healthy Black and White children that undertook a series of lung function measurements which enabled an evaluation of the differing methods of quality control and interpretation and the subsequent development of recommendations for their use in children. Furthermore by recruiting a large group of children with SCD of the same age it was possible to apply the tests and ascertain the clinical usefulness of these measurements in this disease group. A major limitation of the study was the restricted age range (4 to 12 years) which prevented the impact of puberty on lung function being investigated. The strengths and weaknesses of the study are reviewed in detail in the following sections.

9.3.1 Study population

The recruitment of healthy control children was a major strength of the study. Two hundred and fourteen healthy Black children and 186 healthy White children aged four to twelve years underwent spirometry assessments and subgroups of these children underwent the other selected lung function tests. The healthy Black children were either recruited in London schools (with a wide range of socio-economic status) or in the laboratory at ICH or St Louis, USA (primarily consisting of siblings/friends of children with SCD). The healthy White children came from a range of previous studies based at ICH,[16,104,129,144] and were again primarily siblings or friends of children attending Great Ormond Street Hospital for a range of clinical and epidemiological studies, and came from within a 2 hour travelling distance from London. The exclusion criteria were the same for all control children: Children with Cystic Fibrosis or SCD were excluded as were those with a history of asthma (doctor diagnosis or current (within last 3 months) bronchodilator therapy); prematurity (less than 37 completed weeks of gestation), previous hospitalisation with a respiratory complaint, or a past history of pneumonia, tuberculosis or whooping cough. Measurements were postponed if the child had had a respiratory infection within 3 weeks of the appointment. The standardised inclusion/exclusion criteria for all healthy control children and the range of projects from which they came, minimised selection bias and made the results more generalisable.

A limitation of the study population was that ethnicity was simply defined as either “White” or “Black,” and Asian children were not included. Whilst lung function reference data based on height and sex tend to be applicable in White European children of the same age and sex,[126] larger differences in anthropometry have been observed between children of Black African and Black Caribbean backgrounds, with

African children being smaller and hence having a lower absolute FEV_1 than their Caribbean peers.[8] FEV_1 has been shown to be 13-14% lower in Caribbean children and 15-17% lower in African children compared with White children of the same age and sex.[8] These differences have been attributed to anthropometric differences in trunk/leg ratios between these ethnic groups,[8] although chest wall dimensions are also thought to contribute to these differences.[9] Ethnic differences in anthropometry and lung function have also been reported in Asian children, with FEV_1 reported to be around 7% lower than in White children of the same age and sex.[2,8] The inclusion of healthy Asian children however, was beyond the scope of the thesis as a primary aim was to interpret lung function in children with SCD (a disease predominantly associated with Black subjects). Furthermore, whilst dividing the Black children into sub-groups of African or Caribbean origins may have provided more information regarding ethnic differences in growth and lung function amongst Black children, the modest sample size meant there was not enough power to achieve this. The challenges surrounding the impact of ethnicity (and the many different ethnic groups) will be addressed further in section 9.6.

In addition to the healthy children reviewed in this study, a large cohort of children with SCD was also recruited: All eligible children from three London hospitals (St Marys, North Middlesex and St Thomas') were approached, therefore sampling bias was minimised. A detailed respiratory questionnaire and information regarding hospitalisation was documented (see appendix 6 for details), however it was beyond the scope of this thesis to compare extensive clinical details with the lung function assessments (future research directions include more comprehensive investigations of clinical details and lung function assessments during serial studies (section 9.6)). The children with SCD presented with a range of respiratory symptoms (12% had a doctor diagnosis of asthma at the start of the study, however 50% reported cough on most days), thus enabling the lung function tests (and newly developed recommendations for use and interpretation) to be interpreted in conjunction with some basic knowledge of the clinical status of the child.

The study population of both healthy children and children with SCD was a major strength of the study. The healthy control group enabled investigations of current published reference data and guidelines to be undertaken,[1,2,5,39,41,78,97,98,105,126,147] and where reference data and/or guidelines were unavailable or inappropriate they were developed,[128,146] whilst the SCD group enabled the clinical applicability of these measurements and newly developed recommendations to be assessed. The inclusion

of healthy Black children, was particularly pertinent for identifying group differences between health and SCD as significant misinterpretation would have occurred had interpretation been based solely on published reference data from White children,[41,65,97,98,126] even after using the ethnic adjustments suggested by the ATS/ERS.[2] The clinical implications of using reference data based on White children, and the appropriateness of ethnic adjustments are discussed further in section 9.5.

9.3.2 Sample size

Table 8-3 in chapter 8 summarises the number of children who underwent each lung function test at baseline, and describes the magnitude of difference that the study was powered to detect for each test depending on the precise sample size. For all baseline measures, the number of children assessed enabled differences of 0.5 SD (Z Score) to be detected with 80% power at the 5% significance level, hence the significant differences between healthy Black children and predicted values based on White children in IOS outcomes, and the ethnic differences detected in plethysmographic lung volumes and spirometry were adequately powered, whilst the difference of 0.2 Z Scores between healthy Black and White children in sR_{aw} outcomes was not significant. The large sample (1908) of sR_{aw} data from healthy White children meant there was sufficient power to develop reference equations for this outcome.[128]

Reference equations for all other outcomes however could not be developed based on the sample size available since at least 300 local healthy controls (150 males and 150 females) would be needed to validate published reference equations with any degree of certainty, as in smaller sample sizes differences of up to 0.5 Z Scores may arise purely by chance.[156] Although the development of reference equations for IOS and plethysmographic lung volumes was not an aim of the study, the lack of adequate reference equations was a limitation to the interpretation of results.

Since discrepancies between IOS results and the predicted values based on reference data were observed, multiple regression analysis was used to determine the impact of SCD on each IOS outcome. SCD had a significant impact on IOS outcomes with increases in R_5 values, and decreases in R_{15} values of ~0.06 $kPa \cdot L^{-1}.s$ (equating to~ 0.5 SD), and greater differences for R_{20}, Fdr and AX was observed. Thus, IOS was sufficiently powered to detect the group differences observed. However, since there was not enough power to develop reference equations and/or limits of normality, IOS could not be interpreted on an individual basis, as the limits of normality could not be reliably established.

Further limitations in IOS were found when reviewing within-subject, between-test repeatability and bronchodilator responsiveness. Twenty-two healthy Black children underwent between-test repeatability measures, this meant there was 80% power to detect differences of 0.85 SD (0.102 $kPa \cdot L^{-1}.s$ in R_5 values) at the 5% significance level, however there was no significant difference in between test repeatability (mean difference) of 0.0 $kPa \cdot L^{-1}.s$ in health and SCD. Twenty-five healthy Black children underwent measures of BDR. This sample size meant a difference of 0.8 SD could be detected with 80% power at the 5% significance level. The difference in R_5, R_{10} and R_{20} post bronchodilator was ~0.07 $kPa \cdot L^{-1}.s$ (~0.5 SD). This was neither a clinically or statistically significant change. Thresholds for BDR were therefore calculated using Bland and Altman analysis and 95% CI were calculated for both the upper and lower limits of agreement to ensure that conservative limits of agreement were derived. BDR was found to be of similar in both healthy children and children with SCD. Similar numbers for repeatability and BDR were used in sR_{aw}, and a similar level of BDR in health and SCD was observed (i.e. no significant group differences).

BDR assessments were also conducted with spirometry as an outcome measure. The sample size of 50 children assessed meant that a difference of 0.56 SD (equivalent to 6.7% predicted or 22 mL) could be detected with 80% power at the 5% significance level. Whilst statistically significant changes from baseline were observed in both health and SCD, they were not clinically relevant, nor did they meet the thresholds of significance (12% change from baseline and/or 200 mL increase in FEV_1) recommended by the ATS/ERS.[105] Finally there was no significant difference in BDR observed between health and SCD. In summary, despite the limited numbers of children undergoing BDR assessments using IOS and sR_{aw} outcomes, an adequate number of children underwent BDR assessments with spirometry. All three lung function assessments demonstrated a similar extent of BDR in health and SCD, therefore demonstrating that BDR is not a common feature in those children with SCD assessed in this study.

9.3.3 Anthropometry

Anthropometric differences between Black and White children are known to exist.[8] In particular the trunk: leg ratio has been shown to be higher in White European children in comparison to children of Black origin, resulting in lung volumes being larger in White children compared to Black children of similar sex and standing height.[4-8,150,157] The current study demonstrated that Black children were taller, heavier and had a higher BMI when compared with White children of the same age (Figure 3-1). Given the known anthropometric differences between Black and White children and the potential impact this has on lung function, it was essential to express anthropometry on a common scale (i.e. as Z Scores) which adjusted for differences in sex and age. An advantage of this study was that it provided an opportunity to evaluate the anthropometric reference data available for children of different ethnic origins.

In the UK, the British 1990 anthropometry reference equations based on White children are commonly applied,[130] whereas the USA commonly use the CDC 2000 growth charts[131] which include Black, White and Hispanic children and therefore represent an average from all these children. Regardless of the equation applied, the mean height, weight and BMI Z Scores in the healthy children under review were significantly higher than the expected value of zero Z Scores (White children centred around 0.3 Z Scores, whilst Black children centred around 0.8 Z Scores). This offset from zero had significant implications in defining the limits of normality for growth in children of this age group. For example a “normal range” (defined as mean+/-1.96 SD) of -1.66 Z to +2.26 Z for White children, and -1.16 Z to +2.76 Z for Black children may be more suitable (see section 3.2.3). Without the control group, growth restriction in children with SCD would have been missed as their values centred around zero, yet were significantly lower than the Black controls.

Statistically significant mean differences between the two anthropometric equations were observed (mean difference ~0.1 Z Scores). Although these differences may have minimal clinical significance, the Bland and Altman analysis revealed clinically significant wide limits of agreement (~1 Z Score) and a positive bias, with Z Scores calculated by the British equations[130] generally being higher than those calculated with the CDC equations.[131] The largest discrepancies between values predicted by the two equations were observed in Black children, who had significantly increased height, weight and BMI Z Scores compared to their White peers. The discrepancies in Z Scores between the two equations were not surprisingly more prominent in the Black children when using the British 1990 equations,[130] since these were based entirely on

White children. The inadequacies of expressing anthropometry based on White reference data have been described before by Wang *et al*.[158] These findings highlight the importance of selecting the most appropriate reference data as the two equations were not interchangeable. Whilst the British 1990 anthropometry equations[130] may be adequate for UK studies on White children only (for example Cystic Fibrosis research[104]), multi-ethnic and multi-centre research studies such as the SAC study should use the CDC 2000[131] reference equations, and ensure the same reference data are applied during longitudinal studies. The offset from zero observed in this study should be taken into consideration when attempting to identify either growth restriction or evidence of obesity in children.

It has been reported that White people have higher fat free masses, higher inspiratory and expiratory muscle pressures and wider chests than those from the other races.[159] Slight anthropometric differences between healthy Black and White children were observed despite using equations that included both Black and White children in the reference data, and expressing results as Z scores which adjusted for age and sex.[131] Despite the slight increase in height, weight and BMI Z Scores of ~0.5 Z Scores in Black children, there was no correlation between BMI Z Score and lung function outcomes. The challenges in expressing anthropometric differences between Black and White children may be minimised if lung function is expressed in relation to body size and shape rather than just standing height. Although now standard practice, at the time when most of these measurements were undertaken we did not measure the ratio of sitting to standing height which may contribute to ethnic differences in lung function.[160] Protocols have since been amended to include sitting height in all future lung function assessments and further work including body size and composition studies has commenced (see section 9.6).

Another possible limitation to the study were the slight differences in height Z Scores in healthy Black children studied in the UK compared to those studied in the USA (British children were ~0.4 Z Scores taller) despite identical measuring techniques. This observation may represent real physiological differences between healthy children in the two different countries which could be a consequence of several factors including diet and genetic ancestry (i.e. the difference between Black-British and African-American) which could not be investigated in the current study but will be investigated in the future. Despite these anthropometric differences, no difference in lung function was observed once results were adjusted for height, age and sex.

9.3.4 Age range

Ideally the research questions should have been addressed in a wider age range to encompass children from 3 to 18 years of age. However, practical limitations restricted the age range assessed. The Asthma UK initiative was a collaborative study which collated normative data in children aged 2 to 11 years and the subsequent reference data and recommendations were based on children aged 3 to 10 years, hence the study of contemporaneous healthy Black children and children with SCD had to be limited to a similar age range. Similarly the IOS reference data by Dencker *et al*[41] were also limited to young children less than 11 years of age (the Nowowiejska equations,[65] which extended to 18 years, were found to be unreliable (chapter 5)). Finally, there was an opportunistic approach in combining healthy children recruited for the SLIC study (which was limited to primary school children to minimise education disruption). While results from this study are not applicable to adolescence where considerable growth and development of the respiratory system occur,[3] data describing ethnic differences in lung function during adolescence are available.[157] We therefore focussed on age groups where measurements were most challenging and information most lacking.

The lower age limit in the study differed depending on the lung function test studied, hence interpretation of some pre-school lung function was limited. Objective assessments of lung function in the pre-school years are important for understanding normal growth and development of the respiratory system as well as the evolution and natural history of disease processes. Furthermore, lung function has been shown to track throughout childhood,[161] hence early identification of lung abnormalities are essential for optimising clinical management. IOS and sR_{aw} measurements included children as young as four years of age. Spirometry has been demonstrated to be a feasible outcome measure in pre-school children,[1,83] and was successfully undertaken in all children greater than four years of age. However, interpretation of spirometry in children less than six years of age was not possible because of the lack of ethnic specific reference data (Wang equations reported results down to six years only[5]). Similarly, despite the apparent successes of some groups obtaining successful plethysmographic lung volume measurements in pre-school children,[162] it was not attempted in children less than six years of age due to the technically challenging nature of the test, especially when a complex protocol was being undertaken. Black pre-school children assessed in this study therefore only undertook two of the four investigated lung function tests (IOS and sR_{aw}). Neither measurement proved useful in the assessment of children with SCD.

Spirometry results from children aged six years upwards in this study revealed significant differences between healthy children and children with SCD (FEV_1 and FVC were significantly lower amongst children with SCD). Spirometry may yet prove to be the most useful test to assess pre-school children with SCD, but this cannot be ascertained until the Global Lungs multi-ethnic equations are released (www.lungfunction.org). Until that time a temporary solution was proposed in terms of using an ethnic adjustment of 0.85 for FEV_1 and 0.87 for FVC (i.e. dividing the results by 0.85 or 0.87 respectively before applying the All-Age Stanojevic equations).[126] Bland and Altman analysis revealed no bias among the older subjects studied between percent predicted results calculated according to Wang *et al*[5] and those calculated from the All-Age[126] (adjusted) equations. Results would, however, need to be interpreted with caution especially when switching to Wang equations at six years of age due to the known potential problems which may occur when switching between reference equations.[151,153,154]

The upper age limit for the study of the healthy children was 12 years. With the exception of sR_{aw}, which demonstrated minimal age-related changes, the upper age restriction limited interpretation in all measurements. In IOS significant frequency dependence of resistance (Fdr) was observed in both health and SCD, however it was more pronounced in children with SCD. Fdr is generally associated with peripheral airway obstruction in adults,[124] however Fdr is also a common feature in healthy young/small children since it reflects the relative increase in peripheral airway resistance compared with resistance in the more central airways. This may be a reflection of the dysanaptic growth occurring in which lung volume (alveolar growth) increases at a greater rate than airway size,[3] however the magnitude of flow/pattern of flow and the involvement of turbulent flow can also contribute to the differences between peripheral and central airways resistance. Several studies (based on White subjects) have demonstrated that significant Fdr occurs naturally in healthy young children, however by ~13 years IOS resistance values are similar to that found in adults, with no evidence of Fdr in healthy adults.[38,51,52,57] Since anthropometric differences were observed between both healthy White and Black children, and healthy Black children and children with SCD, it was not possible to determine whether the Fdr observed in SCD had some pathophysiological basis (i.e. greater peripheral airway obstruction) or was simply a function of these anthropometric differences. The lack of appropriate reference data for IOS across the ages and ethnicities was a further limitation of this measurement.

There was no relationship between the calculated Z Scores for spirometric or plethysmographic outcome measures and age in the healthy children assessed suggesting the reference equations applied were appropriate across the age range studied (6 to12 years). The important physiological changes that occur during the pubertal growth spurt, and their potential implications on lung function, could not be assessed in the current study, but have been described recently.[3] Quanjer *et al* compared FEV_1/FVC ratio against age in 22,412 healthy subjects aged 4 to 20 and found a "kink" at puberty, which was thought to reflect changes in chest dimensions and respiratory mechanics during puberty.[3] This age dependence of lung volumes was also observed in RV/TLC, and similar patterns of growth and development were observed across different ethnic groups, however there was a notable offset, highlighting the importance of using ethnically appropriate reference equations. Although the reference equations used in the current study appeared to be adequate (with the exception of FRC which required some adjustment factors), the applicability of these equations in older children, where rapid changes in airway properties, body proportions, thoracic shape and respiratory muscle function are occurring could not be assessed. Furthermore it was likely that some of the children in this study would have already entered puberty. Although pubertal staging was not included in the original part of the protocol, future studies will include Tanner stage pubertal questionnaires[57,58] and further comparisons of teenage children and investigations into the relationships between lung and somatic growth are currently being undertaken (see section 9.5.2.1).

Despite the limited age range, results from the study improved the application and interpretation of the selected lung function tests in young children. Whilst the interpretation strategies are limited to the age-range studied, recommendations for applying each lung function test may prove to be a useful starting point for developing recommendations which encompass all ages.

9.3.5 Study protocol

A major strength of the study was the standardised protocols used for all measurements which enabled direct comparisons between healthy children and children with SCD and ensured that any differences detected were real physiological differences, and not simply a consequence of differing methodology. The following sections describe the strengths and weaknesses surrounding the study protocol, paying particular attention to the quality assurance that enabled inter-centre comparisons together with justification for why specific lung function tests were selected, while others were omitted.

9.3.5.1 Quality assurance

The stringent quality assurance applied to all measurements was of considerable advantage for this study. Where guidelines, standards or information were missing, investigations were conducted so that new or amended guidelines could be developed. Strict adherence to protocol has been a policy at ICH for several years, and therefore enabled data from healthy White children previously studied at ICH to be included in the comparisons without creating a bias.

The importance of biological controls, and standardising equipment and internal settings was demonstrated by Poorisrisak *et al*, who recruited seven young children aged 4.9 to 6.6 years to act as biological controls to repeat plethysmographic measurements in six centres and found sR_{aw} results to be significantly lower in two centres due to a difference in factory settings.[163] Although it was not possible to have international biological controls, within-centre adult biological controls served as a verification of equipment accuracy, and a central over-read process enabled a rapid response to any potential equipment/protocol problems. In contrast to a recent report which found subtle inter-centre differences in lung volumes despite identical protocols,[164] no between-centre differences were detected in this study, despite the use of different plethysmographs (UK utilised Jaeger, whilst the USA utilised the Sensor Medics body box).

Anthropometric differences in healthy children studied between centres were observed despite identical measurement protocols, equipment and regular calibration. After making adjustments for height, age and sex, there were no differences in lung function amongst the healthy children assessed in different centres, thus suggesting the observed anthropometric differences reflect real population differences rather than differing measurement techniques. The importance of accurate measures of height

and of expressing anthropometry in Z Scores using appropriate reference data, was recently highlighted by Quanjer *et al* who recently re-calculated predicted FEV_1 and FVC values from 26,321 healthy White children (51% male) aged 6 to 19 years using whole numbers for age (years) and height (cm) compared with decimal age and height. They demonstrated that inaccurately entering age and height into prediction equations led to considerable additive errors in predicted lung function values such that a 1 cm error in height resulted in errors in spirometric outcomes varying between 1.2 and 3.0%.[153] All stadiometers underwent regular verification checks with a standard measuring rod, and staff were trained to ensure accurate measures of height were obtained.

9.3.5.2 Inter-centre comparisons

Results from this study demonstrated that with strict adherence to protocol, within-centre biological controls and prospective over-reading with rapid feedback; there was no bias between results collected in different laboratories in the USA and UK, and between spirometry results collected in London schools when compared to those measured in a specialised paediatric lung function laboratory. The inter-centre comparisons proved to be vital in demonstrating the generalisability of the results, and added confidence that results obtained in this study can be applied in other centres, providing protocols are adhered to.

In addition to the prospective data collection, and inter-centre comparisons (London school Vs. London laboratory Vs. St Louis Laboratory (section 7.6.1)), retrospective sR_{aw} data collated from five international centres were compared as part of the Asthma UK Initiative to develop reference equations. This was the largest collation of sR_{aw} normative data to date. The advantages of this study were that it allowed different methods of data collection and analysis to be scrutinised, and consensus recommendations to be developed. The disadvantage of this study was that the retrospective nature of the data collection meant that two centres had to be excluded due to methodological differences. Interim recommendations and reference equations were however developed which were important for the prospective data collection which followed, and further recommendations will be made in the near future (see future work, section 9.6).

9.3.5.3 Limitations of the study protocol

The study protocol was devised by the SAC team and took approximately 90 minutes to undertake the lung function assessments in addition to the study questionnaire and included assessments of children with SCD and healthy Black children. Of the four lung function tests under investigation, IOS was the only assessment which was new to ICH and hence had no retrospective controls (Black or White) to validate the methods. The lack of a White control group limited the interpretation of IOS results in Black children since the differences observed could not be proved to be due to ethnic differences alone. IOS was part of the SAC protocol which originally only assessed children with SCD. A further limitation of IOS was associated with data management. Despite the potential usefulness of IOS in differentiating asthma from healthy White children,[45,47,67,68] the limitations surrounding data extraction and reference data means that IOS is not considered to be a useful clinical test for children with SCD at the present time.

In addition to the lung function assessments evaluated in this thesis, the children with SCD also underwent echocardiograms, over-night sleep studies, methacholine challenges, and blood tests (results not included in this thesis). Whilst extending the lung function assessments to include multiple breath washout technique (MBW), resistance measured by the interrupter technique (R_{int}), Diffusion capacity of the lung with carbon monoxide (DL_{CO}) and measurements of exhaled nitric oxide (eNO) may have enhanced the clinical picture, lengthening the protocol was not feasible. Justification for the selection of the four lung function tests which were investigated can be found in the introduction (section 1.11). Ideally measurements of $DL_{CO,}$ and K_{CO} (gas transfer per unit lung volume) would have been included as they have been shown to distinguish between healthy Black children and SCD,[165] however the technique is difficult to perform in children less than eight years, and paediatric guidelines are limited.[120] Furthermore, reference data have been shown to be inappropriate in White children[16] and ethnic differences have yet to be delineated. It was beyond the scope of this thesis to develop new guidelines and reference data for this technique.

Measures of airway inflammation (e.g. eNO[39,40]) and hyper-responsiveness (e.g. methacholine challenge testing[166]) were not included in this thesis, however a subset of the SCD children had undergone these measurements as part of the SAC study. While over half the children assessed demonstrated significant airway hyper-responsiveness, this was not related to the typical characteristics of asthma (i.e. respiratory symptoms,

markers of inflammation (eNO), positive response to skin tests to aeroallergens, and reduced FEV_1), suggesting a different pathophysiology from common childhood asthma.[167] Whilst performing measures of DL_{CO} and methacholine challenges in children with SCD may elucidate the pathophysiological mechanisms in SCD, these measures were beyond the scope of the thesis which was primarily to improve the application and interpretation of four commercially available lung function tests that are commonly applied in young children.

SCD is associated with repeated lung injury, and further characterised by haemolysis, endothelial cell dysfunction, vascular disorders and leucocytosis. There is also much evidence that acute chest syndrome (ACS) is associated with a reduction in lung function,[168] with repeated episodes of ACS resulting in scattered areas if lung fibrosis,[169] and evidence of restrictive lung defects in adults.[169,170] Although details regarding these symptoms were collected as part of the SAC study, these details were not available at the time of writing this thesis and will be reviewed in the future. Despite the limitations described previously, this study described appropriate measures of data collection and analysis and, by standardising these methods, proved that these methods can be reliably applied in other centres.

9.4 Comparison of results from current study with previous literature

The overall aim of the thesis was to improve the application and interpretation of paediatric lung function tests. The following sections compare the results obtained in this study with those published in the literature and focuses on published guidelines/recommendations, and normative data for each lung function test. Repeatability and the bronchodilator response for each outcome are then evaluated followed by a comparison of the lung function results obtained in the SCD children studied for this thesis with those reported in elsewhere.

9.4.1 Impulse Oscillometry

9.4.1.1 IOS: Published guidelines

Impulse Oscillometry (IOS) is a commercially available, effort-independent lung function test which measures respiratory mechanics. Whilst there are some published guidelines available which generally describe how to optimise data quality by observing the real-time volume/time display to identify data corruption (i.e. airflow leak due to talking, chewing, swallowing etc),[1,39] there is no international consensus on quality control.[38] Some research groups exclude entire measurements if there was any evidence of airflow leak[36] whereas others suggest editing/selecting “acceptable” segments.[41] The current study developed an over-read criteria based on the literature and personal correspondence from the late Dr Michael Goldman. Results from the majority of children met the specified criteria, however since the physiologists were specially trained to perform online QC (i.e. coaxing the child) and were aware of the QC criteria that would be applied after the measurement, our ability to assess the true impact of applying such criteria to more naïve investigators was somewhat limited. It is expected that fewer children would meet the QC criteria, or data would have required more editing had the measurements been undertaken by less experienced physiologists.

A comparison of results before and after carefully applying the QC criteria revealed no difference in the results, and just a small reduction in the within-test SD. Whilst minimising within-test SD is potentially useful for research studies, the small difference to SD observed in the current study was probably not clinically relevant, and the time spent applying this quality criteria (about 10 minutes per measurement session) would not be clinically feasible for routine use. The overall recommendations for performing IOS based on the current study and the published literature are to ensure

measurements are undertaken by an experienced, trained operator and undertake as much online QC as possible (i.e. coaxing the child into a gentle breathing pattern as described in the methods section 2.6.4.4).

9.4.1.2 IOS: reporting results

A secondary aim when reviewing IOS was to describe the most appropriate method of summarising the results and to ascertain appropriate outcome measures. Whilst the average of three[44] to five[171] IOS measurements (i.e. up to 90s continuous recording of tidal breathing) have been suggested, the current set-up of IOS software displays just one measurement at any given time, and subsequent attempts are stored as separate measurements thereby limiting the ease with which reports can be generated. Reporting the median of three measures was the simplest and fastest method of reporting IOS results as it was relatively easy to select the "middle" result of three (or five) measurements, and no statistical differences ($p<0.0001$) were observed between reporting IOS outcomes as mean or median of three measures.

IOS outcomes can be measured at several different frequencies (5-35Hz), with the general assumption that high frequency oscillations (e.g. $\geq$20Hz) remain in the upper airway and hence reflect resistance in the more central airways, whereas low frequency oscillations (5Hz) can be transmitted through the upper airways towards the lung periphery and therefore reflect a combined resistance of the peripheral and central airways.[38] Whilst this potentially provides useful information regarding different regions of the respiratory system, and it is common to describe resistance and reactance at discrete frequencies,[67,172-174] more informative outcomes may be those which describe the difference between R_{20} and R_5 (Fdr_{5-20}) and the area under the reactance curve from X_5 to F_{res} (AX). Where Fdr_{5-20} may help to discriminate peripheral resistance from the central resistance (as R_5 reflects information on both central and peripheral airways), and AX represents an average of all the low frequency reactance applied, both outcomes have the advantage of exaggerating subtle changes in resistance or reactance at different frequencies and therefore represent more discriminative outcomes. These outcomes have been used in a few recent studies[124,175,176] and have been proposed as the better indicators of uncontrolled asthma, compared to resistance measured at discrete frequencies.[176] In the present study Fdr_{5-20} and AX identified larger group differences between healthy Black children and children with SCD than when reporting resistance and reactance at discrete frequencies, and were more repeatable measures. Fdr and AX were therefore suggested as the preferred IOS outcome measures.

9.4.1.3 IOS: Normative data

To date five IOS reference equations have been published,[41,62-65] none of which were based on Black children. When attempting to select the most appropriate reference equation to apply, the Chinese[63] and Iranian[64] reference data were excluded from the outset as they used linear stepwise multiple regression to develop the reference equations i.e. separate equations for each year group which potentially introduces errors when "progressing" to another age/reference equation.[177] In addition, the authors specified that these equations were "suitable for children in their local area" and hence they were not considered to be appropriate for the Black population in this study. Since reference equations by Frei *et al*[62] were considered too restricted in terms of height range (100-150cm), only two equations based on White children were reviewed.[41,65] Discrepancies between these two equations meant they were not interchangeable. Despite divergences from the expected mean zero Z Score observed in the healthy Black children, the investigations suggested that the equations by Dencker *et al*[41] were the more appropriate, although still limited by the lack of equations for AX, and Fdr. Furthermore the lack of IOS data from healthy White children prevented the impact of ethnic differences to be determined.

As described earlier, Fdr is significant in early childhood,[51,52] however, by ~15 years of age all Fdr disappears and resistance in a healthy adult is considered to be constant (~0.25 $kPa \cdot L^{-1}.s$) at all frequencies.[178] For this reason, many adult studies make direct comparisons between absolute results from subjects of the same age without expressing results as Z Scores.[38,179] Comparison of absolute values rather than Z Scores in children is however, inappropriate because of the known age-dependent Fdr. An example of this inappropriate interpretation comes from Shi *et al*, who, despite describing significant correlations between IOS outcomes and height and age, and referencing the five reference equations described above, chose to interpret results based on absolute values merely stating "caution should be exercised in using absolute values for cut points in children who differ in age or height."[176] Despite the limitations of currently available IOS reference data, an effort to express results as Z Scores (and therefore make some adjustment for differing height and age) should be made.

IOS is considered to be less technically demanding than spirometry, and has been shown to discriminate asthmatic children from healthy children.[45,47,67,68] It is also argued that IOS provides more information about the peripheral airways which is not provided by spirometry. However, given the limited use in SCD and the fact that success rates are similar to spirometry, its use in clinical practice remains doubtful.

The current study has advanced the field of impulse oscillometry measurements in children by:

- ***Developing recommendations for the application and data reporting of IOS measurements***
- ***Highlighting the inadequacies of current reference data.***

9.4.2 Specific airways resistance

9.4.2.1 sR_{aw}: published guidelines

Plethysmographic Specific Airways Resistance (sR_{aw}) is a measurement of airway resistance corrected for lung size, which is measured during tidal breathing from the relationship between simultaneous measurements of airflow and the change in plethysmographic pressure (as a reflection of alveolar pressure) without the need for any special breathing manoeuvres against an airway occlusion.[77] The equipment is commercially available and the feasibility of applying this effort-independent measurement in young children is very good.[78] No children participating in this study were excluded due to “unacceptable” results.

Prior to the inception of this thesis, there were no official guidelines for sR_{aw} measurements, consequently, reported values of sR_{aw} had been collected under a variety of differing measurement conditions such as use of a re-breathing bag or panting technique to achieve body temperature, pressure, and water vapour saturated (BTPS) conditions[80] versus quiet tidal breathing with subsequent electronic compensation for changes in temperature and humidity throughout the breathing cycle;[79] modified masks[82] vs. mouthpieces;[83] use of bacterial filters or not; and a variety of breathing patterns and frequencies.[81] Furthermore differences in selecting/modifying the pressure-flow loop, methods of reporting[86,87] and the use of different outcome measures for sR_{aw} were apparent.[78,180-182] The current study collated and evaluated sR_{aw} data from five international centres which all used the same equipment (Jaeger, Carefusion).

Recommendations for standardising measurements of sR_{aw} in children were developed.[128] These included using equipment that encompasses electronic compensation, (since methods of achieving true BTPS conditions are largely redundant in the modern age), and using bacterial filters with appropriately sized mouthpieces and

noseclips (rather than a modified mask[82]) since these are now used routinely for many preschool lung function tests[1] as well as in older children. Although not undertaken as part of this study, direct comparisons between results obtained with a modified mask and mouthpiece found no difference,[145] findings that were supported by the similar values of sR_{aw} (within 0.1 kPa·s) between centre 4 (mouthpiece) and centres 2 and 5 (mask) in the current study. Previous studies in children have reported statistically significant, though clinically unimportant, alterations in spirometric flows and interrupter resistance when filters are used.[183,184] To adjust for the potential change in resistance provided by a filter, the Jaeger equipment has a checkbox in the internal settings to confirm a filter is *in situ.* Although the effect of filters was only investigated in a relatively small group of adults in this study (see appendix 3), there was a clear increase in sR_{aw} with the filter *in situ*. With an average FRC of 3.1L in these adults and a mean increase in sR_{aw} of 0.19 kPa·s, this equated to a change in R_{aw} of 0.06 $kPa \cdot L^{-1}.s$, which was in keeping with the manufacturer's claim that bacterial filters add no more than 0.1 $kPa \cdot L^{-1}.s$ to the resistance (Air Safety LTD, Lancashire, UK). Nevertheless, the impact on sR_{aw} equated to 1 SD of between-subject variability in health which could result in overestimation of sR_{aw} and potential over-diagnosis if filters are used without adjusting the internal settings to specify for a filter *in situ* in patients with lung disease (as most infection control policies now dictate), but not in the healthy controls from whom reference data are derived.

One of the most significant findings of the sR_{aw} study was the impact of changes in breathing pattern and airflow on the results. It has been suggested that breathing frequency can have a marked impact on measured values of $sR_{aw,}$[81] thus all collaborating centres adhered to the sR_{aw} recommendations for breathing frequency (30 and 45bpm). However, an elevation of breathing pattern in terms of flows attained and breathing frequency demonstrated that the majority of children aged 4 to 11 years adopted a breathing pattern which had a netflow $<1.5L \cdot s^{-1}$ and breathing frequency <50bpm, and results suggested that healthy children may breathe at higher breathing frequencies without increasing sR_{aw} values.

9.4.2.2 sRaw: reporting results

Various outcome measures and the method of reporting these were investigated. Whilst sR_{eff} was the preferred outcome because it is calculated as a regression of pressure and flow over the entire breathing cycle and may therefore provide more information than sR_{tot} (calculated between points of maximum plethysmographic (box) pressure) it was demonstrated that they were highly correlated and that one could be predicted from the other. Given that in the past different research centres have reported sR_{aw} as either sR_{eff} or sR_{tot} [83,129,141] and the limited evidence as to which is the most discriminative in various disease processes, it was recommended that both outcomes be reported for the foreseeable future.

9.4.2.3 sRaw: Normative data

Part of this thesis was dedicated to the development of sR_{aw} reference equations (created by Dr Sanja Stanojevic).[128] The new equations, however, included the caveat that they could only be applied to populations that had been measured using the same methodology and similar demographics (e.g. White children aged 3 to 10 years), and were for these reasons an interim solution. Nevertheless, these preliminary equations were far more appropriate than those currently available in Jaeger equipment. The "Jaeger-kids" for children aged 4 to18 years, and "Jaeger" for those >18 years were based on data collected under BTPS conditions over 30 years ago[80] and have identical predicted values for sR_{eff} and $sR_{tot,}$ whereas we found sR_{eff} to be significantly lower than sR_{tot}. Furthermore the 'Jaeger-kids' predicted values of 0.51 kPa·s for girls and 0.53 kPa·s for boys <18 years significantly under-estimated the actual values observed in healthy children in this study, which were collected using electronic compensation. If the interpretation had been based on the default reference data within the equipment, serious over-estimation of the degree of airway obstruction in children with lung disease would have occurred. Whilst we observed a very gradual decline in the predicted values with age, the Jaeger equations suggest that there is a sudden (and physiologically implausible) *increase* in predicted values to 0.96 kPa·s for females and 1.18 kPa·s for males from 18 years of age onwards.

In contrast to other lung function outcomes, ethnic group did not appear to influence measures of $sR_{aw.}$ This lack of ethnic differences in sR_{aw} has been reported previously for Asian preschool children[185] and was an expected finding since sR_{aw} is the product of FRC and R_{aw}, and therefore internally adjusts for any ethnic differences in resting lung volume. sR_{aw} could therefore be interpreted with some confidence in children with

SCD. To date there are no other published studies of young children with SCD undergoing sR_{aw} measurements.

This study advanced the field in sR_{aw} measurements in children by:

- ***Providing the most comprehensive guidelines and reference equations to date.[128]***
- ***Demonstrating that ethnic differences in sR_{aw} do not exist.***
- ***Providing confidence that sR_{aw} data can be reliably applied and interpreted in young Black children using the recommendations and reference data developed.[128]***

9.4.3 Plethysmographic Lung Volumes

9.4.3.1 Lung Volumes: Published guidelines

Plethysmographic lung volume assessments measure the total volume of gas in the lungs at end expiration, including any gas trapped behind closed airways and are the gold standard for diagnosing restrictive lung disease.[2] The full assessment protocol can be found in the methods section 2.6.6. Given the challenging nature of breathing against an occlusion, the measurement is rarely performed below the age of six years.

International guidelines for the application of lung volumes are based on adults,[94] and no modifications to these guidelines have been made to adjust for potential differences in children. The evaluation of the applicability of these international standards in children undertaken in this thesis found that total over-read scores were similar in health and disease, and across the age range assessed, therefore no modifications to these standards were made. These results supported findings by Vilozni *et al* who investigated the feasibility of performing plethysmographic lung volumes in very young children (aged 3 to 7)[162] and found that adult acceptability guidelines were sufficient for this age group, with a reported 70% success rate.[162] Adequate training time (Vilozni reported~15 minutes to teach the child the complex techniques), equipment modifications (such as adjusting the chair height and ensuring the flow sensor was accurate at low flows) and reference data in very young children are the main keys to accurate measurements and appropriate interpretation of results when using this technique.[162] Given the use of lung volumes in identifying restrictive lung disease, particularly in SCD, further work to establish ethnic-specific normative data is warranted.

9.4.3.2 Lung Volumes: Normative data

Lung volume reference data in children are limited and ethnic differences are not well defined.[2] Although plethysmographic lung volumes are considered to be the gold standard lung function test when diagnosing restrictive lung disease,[2] interpretation was shown to vary widely depending on which equation was applied (i.e. those by Zapletal *et al*[97] (commonly used in the USA) or those by Rosenthal *et al*[98] (currently recommended by the British Thoracic Society)). The equations by Zapletal[97] were considered to be the least reliable of the two reference equations as they were derived from a small sample of White children (86 boys and 87 girls) measured over 40 years ago before international guidelines regarding standardised protocols had been published, and using equipment that is no longer available. Results from the current study were therefore expressed as Z Scores (and % predicted) according to equations derived by Rosenthal *et al*, which were based on 772 *White* children aged 4 to 18 years and modern equipment.[98]

As expected, results from this study demonstrated good agreement with the reference data for RV and TLC in the White children assessed, however FRC was found to be over-estimated by ~9% (i.e. mean % predicted FRC in our sample was only 91%). This discrepancy has been reported previously,[16,99,104] and has important implications since clinical evidence of hyperinflation or gas trapping may be missed unless this bias is taken into account. The difference in FRC observed may reflect a change in protocol during recent years, whereby subjects are no longer required to pant rapidly for airways resistance measurements immediately prior to the airway occlusion for thoracic gas volume manoeuvres, a practice that may, in the past have led to elevated resting lung volumes.

The difficulties in interpreting plethysmographic lung volume data are further complicated when investigating Black children. FRC and TLC were found to be 14% and 6% lower respectively in Black children than predicted by Rosenthal equations, such that measured values would need to be divided by 0.86 and 0.94 respectively prior to calculating % predicted values, whereas RV and RV/TLC respectively were 4% and 10% higher. Ethnic differences in lung function have been described previously,[150] with lung function reported to vary between 10% to 25%.[10] The ATS/ERS 2005 Interpretative strategies for lung function tests suggest ethnic adjustment factors of ~12% reductions for TLC, FEV_1 and FVC and ~7% for FRC in Black subjects,[2] whilst most commercial lung function devices simply apply a fixed ethnic adjustment of 12% reduction on all parameters for Black children. Results from

this study demonstrated that ethnic differences differed in magnitude and direction and were dependent on the outcome investigated. Using the Rosenthal[98] reference equations directly, without an ethnic adjustment would have resulted in an over-diagnosis of obstruction/gas trapping (elevated RV/TLC) in SCD. By contrast, application of the 12% reduction (as recommended by the ATS/ERS[2] and implemented by the equipment) would have resulted in the under-diagnosis of restrictive lung disease (reduced TLC) and over-diagnosed obstruction (raised RV/TLC) in SCD. Although currently based on a relatively small sample size, the ethnic adjustments derived from the healthy Black children assessed in this study appeared to be the most robust method of identifying changes in lung volumes amongst children with lung disease. Given the limitations of ethnic adjustment factors[107] there is an urgent need to establish new plethysmography lung volume equations which span all ages (like the All-Age spirometry equations[126]).

This study advanced the field of plethysmographic lung function measurements in children by:

- ***Providing further evidence that young children can perform the measurements and meet adult standards[94]***
- ***Quantifying ethnic differences for each lung volume outcome[146]***
- ***Preventing under-diagnosis of restrictive lung disease which would have occurred had current guidelines been followed[94]***

9.4.4 Spirometry

9.4.4.1 Spirometry: Published guidelines

Spirometry is a commercially available, effort-dependent lung function test shown to be feasible in children from three years and older in both health and disease.[83,111,186-188] Published guidelines for both adult[105] and pre-school children[1] were available for spirometry at the inception of this thesis, and modifications of these guidelines were made to ensure they were applicable for children assessed in the current study.[111] No child assessed in the current study had unacceptable spirometry, and no further recommendations regarding quality control and repeatability for spirometry were made as a result of this study. Although data were not scored, all measurements underwent a central over-read process by an experienced respiratory physiologist (JK and PB (St Louis)), and future work will involve scoring spirometry in the manner suggested by Enright *et al*.[106]

The importance of over-reading spirometry was highlighted recently by Hankinson *et al*, who reviewed the appropriateness of the spirometry QC criteria defined by the ATS/ERS[105] by comparing spirometry QC scores derived automatically from a computer with scores determined by an experienced physiologist. Spirometry results from 1456 healthy adults previously accepted into the NHANES[107] dataset were re-reviewed by Hankinson. Whereas the computer rejected around a third of results primarily due to failure of end of test criteria (FET >6s or no change in volume (<25mL) for >1s), the human over-view only rejected 3-5%.[189] These results highlight the potential problems with relying on computers/strict guidelines to determine acceptability of spirometry data and stress the importance of ensuring an experienced reviewer over-reads all results. All spirometry results in this study met the standards described in the methods (section 2.6.7) and are published in *Pediatric Pulmonology*.[111]

9.4.4.2 Spirometry: Normative data

A plethora of spirometry reference data for children are available[151] with the most comprehensive reference data to date being the All-Age equations.[126] For Black children reference data for children aged 6 to 18 years by Wang *et al*[5] are available, additionally NHANES III equations for Black children >8 years[107] can be applied, however those would have excluded a large proportion of the population studied and were therefore not used.

The ethnic-specific spirometry equations by Wang *et al*[5] proved a good fit for White and Black children, with mean results from our healthy children approximating 100% predicted in the age range assessed. The Wang equations are, however, limited to 13 step-wise sex-specific equations for each year of age from 6 to 18 years for each ethnic group. Extrapolating beyond these age-ranges is not recommended,[125] and therefore these equations are not suitable for the increasing number of preschool children now undertaking such tests.[1] A temporary solution to this problem could be to use the Stanojevic equations with appropriate adjustment factors for Black children less than 6 years, albeit with caution due to the known potential problems which may occur when switching between reference equations.[151] A recent letter to the ERJ highlighted the importance of continuous reference equations by describing the case of an 18 year old male with cystic fibrosis who had serial spirometry measurements post lung transplantation.[154] His progress post-transplant had been generally good, with only one instance of severe respiratory compromise aged 17 years when his FEV_1 had dropped to 57% predicted. Upon turning 18 years of age, the default spirometry reference

equations within the spirometer automatically converted from paediatric (Rosenthal[190]) to adult (ECCS[112]) reference ranges, resulting in an apparent clinically significant decrease in FEV_1 percent predicted (82% predicted to 58% predicted) which would normally trigger an urgent admission and invasive evaluation. The observed differences occurred because the paediatric spirometry reference equations used by Rosenthal[190] were based on height alone with different equations pre and post puberty (pubertal break points at 152.5cm for girls and 162.5cm for boys). If a child is particularly short for age, their results may be calculated on the "pre-pubertal" equations, with under-estimation of their predicted value.[154] Given that many children with lung disease have some degree of growth restriction, as found in the current study and elsewhere,[136] the potential for under-estimating predicted (and hence over-estimating percent predicted) values using spirometry reference equations based on height alone (e.g. those by Rosenthal[190]) or step-wise equations which have distinct "break-points" at each age (e.g. those by Wang[5]) limits the use of these equations and highlights the importance of the All-Age[126] reference equations which provide smoothly changing curves to describe the transition between childhood and adulthood and adjust for both height and age.

The current study has advanced the field of spirometry in children by:

- ***Highlighting the challenges of interpreting spirometry in non-White children***
- ***Developing recommendations for interpreting spirometry in Black children[146]***

9.4.5 Repeatability and the bronchodilator response

Within-test repeatability reflects the consistency of the subjects effort (e.g. used to determine if flow limitation is achieved during spirometry assessments); the instability of intrinsic biological factors (e.g. a stable breathing pattern to determine FRC) and the precision of the LFT device used. A strength of the study lay in the fact that each lung function measurement was repeated several times at baseline which enabled the within-test repeatability to be calculated and compared to that previously published.

Within-test repeatability may be expressed in absolute terms, as a within-subject SD or as the coefficient of variation (CV), which is the SD expressed as a percentage of the mean. In IOS assessments, the most repeatable outcome measure was R_{10} which had a within-test CV of ~5%. This finding was much lower than recent findings by Shi *et al*, who suggested that a within-test CV of 10% for R_{10} to be adequate[176] but similar to that reported by Dr Goldman.[43,124] The lower CV reported in the current study, and studies by Dr Goldman *et al*, may be the result of strict QC (which was derived in

consultation with Dr Goldman) and was shown to minimise within-test variability. Careful attention to within-test QC in sR_{aw} and plethysmographic lung volumes also resulted in lower within-test repeatability to that previously reported: Within-test repeatability in sR_{aw} was 7%, compared to the 8-11% described in pre-school children,[54] whereas the within-test FRC repeatability was 90mL, which was much lower than the published recommendations of 150mL in adults[94] and 160mL in children.[149]

Within-test repeatability of spirometry measures was not investigated in the current study as it has been described elsewhere,[105] however all spirometry results included in this study had a within-test repeatability of <150 mL. Recently, Hankinson *et al* evaluated the impact of applying different repeatability measures to spirometry outcomes and found that the mean results were minimally influenced if there were at least 2 measures within 200 mL, however if repeatability was >200 mL (grade D) mean values were significantly reduced,[189] which study supports the findings in the current study.

Within-subject, between-test repeatability in IOS and sR_{aw} was undertaken in a sub-group of healthy children and children with SCD. Knowledge of the between-test repeatability was required for both tests to enable the threshold for a significant BDR to be determined. Within-subject, between-test repeatability was not attempted in spirometry or lung volumes since this was relatively well established in the literature and would have been an unnecessary additional burden on parents and children involved in the study. Furthermore, the repeated deep inspiration required in the spirometry and lung volume assessments potentially would have confounded the IOS and sR_{aw} results as the deep breaths may have fatigued the child or modified the results.

Between-test repeatability in IOS outcomes was similar to that seen in the within-test repeatability (CV~5%), and the same in health and SCD. These results were consistent with another study which evaluated IOS outcomes (R_5, R_{20} and X_5) in triplicate at baseline and after a placebo in 33 asthmatic preschool children (aged 3-6 years) and found the baseline repeatability for R_5 to be 4.1%.[44] Mean between-test repeatability in sR_{aw} was also found to be similar to the within-test repeatability and similar in health and SCD, and much lower (absolute mean (SD) between-test repeatability: 0.08 kPa·s (0.15)) than that previously reported by Bisgaard *et al* (which ranged between 0.19-0.20 kPa·s).[78]

The purpose of assessing between-test repeatability was to evaluate the thresholds for a BDR, whereby a significant BDR was a result over and above that seen in the between-test repeatability. In IOS, healthy children demonstrated significant reductions in R_5 and R_{10} of 15% whilst the thresholds for BDR in Fdr and AX (which reflect subtle changes over a range of frequencies, and hence physiological changes in the peripheral and more central airways) were much larger at 40%. Two children with SCD were identified with a significant BDR by changes in Fdr and AX, this was not detected with any other outcome. The different thresholds for BDR in each outcome reflect the different areas of the lung that are being measured and the intrinsic variability of each outcome. Low frequencies (5-10Hz) penetrate the small airways (defined as bronchioles <2mm in diameter), whereas high frequencies (15-20Hz) remain in the upper airways which have less muscle and are less likely to respond to an inhaled bronchodilator. Unsurprisingly, this study revealed that outcomes that reflect the more peripheral airways demonstrated greater BDR.

The thresholds for the BDR in IOS derived in the current study were slightly lower than that reported in previous studies which found reductions in R_5 post bronchodilator to range from 20-25%[47,48] up to 40%,[135] whilst significant changes in R_{10} have ranged from 15-20%[48] up to 30%.[50] In addition reductions of up to 65% have been described for AX,[37] and up to 50% for Fdr_{5-20}.[176] These higher thresholds however, were based on preschool children who were slightly younger than the children assessed in the current study. Preschool children have greater variability (higher SD) in all lung function outcomes (hence a larger range in a clinically significant change from baseline) and have been shown to demonstrate increased bronchodilator responsiveness.[47]

Bronchodilator responsiveness was also assessed using sR_{aw} as an outcome. Based on the between-test repeatability measures, and after calculating the 95% CI around the limits of agreement (to take into account the small sample size (see section 9.3.2)) the conservative threshold for a significant BDR was estimated as -0.77 kPa·s (45% reduction in sR_{aw}). This threshold was greater than that suggested by Nielsen *et al* who proposed a cut-off level of a 25% decrease in sR_{aw} after bronchodilator administration based on 29 healthy pre-school children.[90] The differences observed in the current study may be the result of more conservative limits of agreement (i.e. calculating 95% CI) and that a slightly different age range was assessed. Nevertheless, there was no significant difference in the level of BDR seen in the healthy children and the children with SCD regardless of the outcome used.

9.4.6 Comparison of LF results in SCD in this thesis to the literature

SCD is a genetic disorder which predominantly affects children of Black African and Afro-Caribbean origin, and frequently results in significant respiratory morbidity.[14] Several pulmonary complications have been associated with SCD, including airway hyper-reactivity, acute chest syndrome, chronic sickle lung disease, pulmonary hypertension, and sleep disordered breathing,[14] with studies also suggesting SCD progresses from an obstructive lung defect in childhood[24] to a predominantly restrictive defect in adulthood.[25] The main lung function tests used in the assessment of SCD are spirometry, lung volumes and measurements of diffusion capacity (DL_{CO}, discussed in section 9.3.5.3). IOS has been used as a secondary outcome in one study which demonstrated an increased R_{rs} that was associated with reduced expiratory flow rates and an increase in the number of ACS episodes,[76] however sR_{aw} has not been used previously as an outcome measure in SCD. This section of the discussion compares and contrasts the main findings of this thesis with that found in the published literature with particular emphasis on spirometry and plethysmographic lung volumes in subjects with SCD.

The principal findings were that children with SCD had significantly lower values of TLC, FEV_1, and FVC, a normal FEV_1/FVC ratio and similar bronchodilator responsiveness compared with healthy Black children of the same age. Despite the relatively young age, results from this study suggested a pattern of restrictive lung disease in SCD, although some concurrent obstruction was also indicated by the raised RV/TLC, and the relative increase in R_5 in the SCD children compared with healthy Black children. These results are consistent with findings by Maclean *et al*, [25] and Sylvester *et al*,[102] who both reported significant differences in FEV_1 and FVC from predicted values (based on NHANES III African-American spirometry reference data[107]), and an unaffected FEV_1/FVC (consistent with restrictive lung disease) in children with SCD.

A recent study by Knight-Madden *et al* on 80 young adults with SCD and 80 ethnically matched controls (mean age: 23 years) also found a high prevalence of restrictive lung disease in SCD,[168] however, 36% of the healthy Black adults measured were also diagnosed with restrictive lung disease. These results suggest that the normative data used (Jamaican dataset) in the Knight-Madden study over-compensated for ethnic differences since it is unlikely that 36% of an apparently healthy population of young Black adults would have restrictive lung disease. This study emphasises the importance of including a control group, since despite the inappropriate diagnosis of

restrictive lung disease in the healthy group, a significantly greater proportion (60%) of adults with SCD were diagnosed with restrictive lung disease.[168] Although the severity of restriction appears to have been over-diagnosed in this study, the use of a control group did identify significant group differences between healthy Black adults and adults with SCD, consistent with the findings in this thesis.

Longitudinal studies are important for determining the pathogenesis of abnormal lung function in children with SCD, however it was beyond the scope of the thesis to explore longitudinal changes in SCD. A few studies have investigated the impact of somatic growth and lung function in children: Maclean *et al* performed at least two spirometry measures on 413 children with SCD. At eight years of age they found 96.5% of the SCD children had normal lung function (confirmed by comparing results to NHANES III reference equations for Black children[107]), 0.9% had an obstructive defect and 2.6% had a restrictive defect. By 17 years of age 81.3% still had normal lung function, none were obstructive and 18.7% were restrictive.[25] Studies by Koumbourlis *et al* also found a decline in lung function over time that developed into a predominantly restrictive defect that was not associated with growth,[191] however the restrictive defect observed in these children was evident at the earlier age of 10 years.[192] Another study included a retrospective review of 79 children with SCD aged 6 to 19 years with at least two spirometry results (mean length of time between assessments: 3.5 years) and demonstrated that FEV_1 increased at a lower rate in children with SCD than the healthy children, in the same way that FEV_1 has been reported to increase at a lower rate in cystic fibrosis.[193] While there was no correlation between the reduction in TLC and age in the current study, the incidence of restrictive lung function has been previously reported to be greater in older than younger children with SCD, albeit findings based on cross-sectional data.[10] Future studies will include longitudinal assessments of lung function in subjects with SCD.

Further pulmonary complications in SCD have been described in the literature: Caboot and Allen reported "convincing evidence that asthma is a significant comorbidity in children with SCD."[14] This suggestion of asthma was because of the high incidence of airway-hyperreactivity (AHR) that has been reported,[14,167,194-196] along with a frequency of respiratory symptoms.[23,197,198] Boyd *et al* reported 17% if their cohort to be asthmatic,[199] however this is a similar frequency to that observed in the general population,[200] and the current study. Furthermore, bronchodilator responsiveness and high levels of expired nitric oxide (FeNO) are cardinal features of asthma,[201,202] however BDR was not detected in the current study, nor in other published

studies,[168,203,204] and FeNO levels in SCD have been reported to be normal.[102,167,168] Thus, although asthma-like-symptoms in SCD have been reported in this study and in the literature, and the current study demonstrated a raised RV/TLC and R_5 (suggestive of obstruction) in some children, the absence of a BDR, reduced FeNO levels and evidence of accompanying restrictive changes (decreased FVC, normal FEV_1/FVC and reduced TLC suggest that there is a different pathophysiological pathway causing elevated AHR than that usually ascribed to typical childhood asthma.

Results from this study suggest a restrictive lung defect, which may be the result of repeated lung injury since the incidence of acute chest syndrome and reduced FVC has been demonstrated elsewhere,[168] however it was beyond the remit of the thesis to investigate the cause of the restrictive lung disease (i.e. if restriction was a consequence of repeated lung injury or more simply a function of restricted growth). These areas will be addressed in the future.

9.4.7 Interpretation of lung function results in SCD

When interpreting lung function, whether or not a result falls outside the lower or upper limits of normality (LLN or ULN) is often of greater clinical significance than the precise percent predicted value. Depending on outcome, these limits of normality may be defined either as those encompassing 90% of the healthy population, in which case the LLN and ULN are based on the 5th and 95% centiles (i.e. ±1.64 SDs) or alternatively encompassing 95% of the population, whereby the LLN and ULN represent the 2.5th and 97.5th centiles (±1.96 SD) respectively. Conventionally, a LLN derived from -1.64 SD is used for outcomes such as FEV_1 and FVC where only reductions in measured values are clinically relevant. By contrast, for outcomes such as FRC or RV/TLC where either reduced or elevated values may be clinically significant with respect to defining restrictive or obstructive lung disease, then the 95% limits should apply. When results are expressed as SD (or Z) scores, it is self-evident as to whether or not a result lies outside these limits, however interpretation of results when expressed as percent predicted is more complex due to the wide range of between-subject variability (i.e. SD) according to outcome.[32,151] The current study calculated limits of normality based on 95% limits of normality (+/-1.96 SD) regardless of the outcome (i.e. including spirometric outcomes) as this gives more conservative limits of normality and minimises over-diagnosis. The use of 95% limits of normality is common practice when interpreting lung function in paediatrics.[1,16,111,144]

A variety of interpretation methods have been employed when interpreting spirometry in Black children. The majority of studies on SCD have used the ethnic-specific NHANES III reference equations[205] to interpret spirometry,[24,25,191,206] however these equations were not suitable for the young children assessed in the current study as they did not extend lower than eight years of age. The Quanjer reference equations (based on White children)[207] with a 12% reduction to account for ethnicity has also been used,[208] but this method of interpretation was also inappropriate since the current study demonstrated that ethnic adjustments of 0.87 and 0.9 for FEV_1 and FVC respectively would be more appropriate. The use of the Wang equations[5] was investigated and found them to be adequate for the children assessed in this study, however results were then limited to being expressed as percent predicted (with varying limits of normality) and could not be interpreted in children under six years of age. The interim solution for children <6 years was to use the All-Age equations[126] (based on White children) with an ethnic adjustment.

The use of the All-Age reference equations[126] plus an ethnic adjustment of 12% was used in a recent study by Tassel *et al*. They studied 184 children with SCD (mean age 12.6 years) and expressed results as Z Scores using -1.64 Z to define the lower limit of normal.[203] With this method of interpretation, Tassel *et al* found that the prevalence of obstructive lung disease was only 5% which was much lower than that previously reported.[76,192] The lower rate of obstructive lung disease in SCD was accredited to the fact that they used the All-Age equations with appropriate lower limits of normal. They reported that had they used the <80% predicted to define obstruction the frequency of obstruction in their cohort would have risen to 20%.[203] Although the method for identifying obstruction may have been appropriate, the use of an ethnic adjustment of 12% potentially under-estimated the degree of restriction (reported as 14%),[203] since we found an adjustment factor of 10% for FVC and 6% for TLC to be more appropriate.

Interpretation of lung volumes in children to date has been challenging. This study demonstrated differences between two commonly used paediatric reference equations[97,98] and recommended reference equations by Rosenthal *et al*[98] with ethnic adjustments to be the most appropriate interim method for reporting lung volumes in children with SCD. The results were consistent with findings by Sylvester *et al*[7] who evaluated the interpretation of lung volumes in 80 Afro-Caribbean children aged 4 to 17 years. Predictive values based on standing and sitting height and 90% or 77% of lung volumes predictive values from Rosenthal[98] and Cotes[209] were evaluated in these children and the authors found that healthy Afro-Caribbean children had significantly

different lung volumes than those values predicted from White reference ranges based on standing height, with the magnitude of the difference dependent on the reference equation used.[7] Sylvester *et al*, conducted further investigations to assess lung function in children with SCD, however they used their own healthy control data to make group comparisons rather than attempt to use published data with unconfirmed/unreliable ethnic adjustments.[7,10]

An interesting example of possible misinterpretation of lung volumes in SCD was a study by Maclean *et al* who used the 1969 plethysmographic Wang equations[210] (based on height and sex) without ethnic adjustments to interpret lung volumes "as validated race-corrected lung volume prediction equations were not available."[25] The lack of any ethnic adjustment may have over-estimated restrictive lung disease slightly (as we found ethnic differences of ~6%), and the adjusted threshold to define restriction of TLC<70% predicted used in their study (rather than the 80% cut-off suggested by the ATS/ERS taskforce[2]) potentially over-estimated restriction further. In addition Maclean *et al* reported RV/TLC in absolute values and suggested that the "decline" in the mean RV/TLC ratio at 8 years of age to 17 years of 0.3 to 0.21 respectively was supportive of early injury or inflammation resulting in progressive changes in lung volumes across age.[25] Their conclusion is not correct, as changes in RV/TLC during childhood are a consequence of considerable changes in airway properties, body proportions, thoracic shape and respiratory muscle function that occur during growth.[3] Hence the results were unlikely to represent the progression of lung disease from an obstructive to restrictive lung disease, moreover, they simply reflect the change in RV/TLC that occurs with growth. The study by Maclean *et al*,[25] and Sylvester *et al*[26,86] emphasise the importance of using a healthy control group and highlight the need for ethnically matched prediction equations in clinical practice.

9.5 Implications of findings

The overall findings of the study demonstrated significant ethnic differences in spirometric and plethysmographic lung volumes between healthy Black and White children, differences in IOS results obtained from Black children compared to predicted values, but no ethnic differences in sR_{aw}. Furthermore, significant group differences in TLC, FVC and FEV_1 between healthy Black children and children with SCD were observed. The main implications of these findings are that considerable misinterpretation of lung function results would have occurred had it not been for the inclusion of the control group and the "adjustments" or acknowledgement of ethnic differences in lung function that were made. This study highlighted the importance of

measuring healthy children and has important implications on the clinical applications and interpretation of these lung function tests.

9.5.1 Importance of controls

The use of healthy White and Black control children enabled lung function methods and published reference data to be evaluated and, with the exception of spirometry, identified serious limitations in current paediatric lung function reference data. Two paediatric reference equations for IOS were evaluated however neither was suitable for interpreting IOS data obtained from Black children (assessment in White children was not performed, see section 9.3.5.3). Had the Dencker[41] equations (based on White children) been applied directly to the IOS results from the SCD children, all results would be considered to be within the normal limits (+/-1.96). However by comparing the results to the healthy Black children of the same age, significant differences both in absolute terms, and in the pattern of results (i.e. frequency dependence of resistance) could be identified. Although IOS assessments could not be interpreted on an individual basis because of the lack of defined limits of normality, the inclusion of a healthy control group enabled group differences to be detected.

The collation of sR_{aw} data from healthy White children was the primary aim for the Asthma UK Initiative and enabled the development of the most comprehensive reference data for sR_{aw} to date,[128] thus study of healthy control children were imperative to improve interpretation of sR_{aw} measurements. Moreover, it was essential that prospective controls (both White and Black children) were recruited to investigate the various factors that influence sR_{aw} results and to confirm that ethnic differences do not occur. sR_{aw} did not prove to be a useful outcome measure when investigating lung function in SCD, however, the findings in this thesis mean that sR_{aw} can be reliably interpreted in Black children with suspected lung disease.

Further evidence of the importance of measuring healthy control children came in the assessment of plethysmographic lung volume measurements. The two paediatric reference equations were evaluated and interpretation of lung volumes was shown to vary widely depending on which equation was applied. The equations by Rosenthal *et al*[98] were the more appropriate of the two (since they were based on a larger sample size and on modern equipment), however there were still inaccuracies with estimating the predicted FRC values in White children. Had the healthy White children not been studied, the extent of hyperinflation (elevated FRC) would potentially have been mis-diagnosed in these children. Furthermore, use of healthy Black controls added to the

evidence that ethnic differences in lung volumes exist, but demonstrated that magnitude and direction of the difference were dependent on the outcome investigated. Had standard guidelines for interpreting plethysmographic lung volumes been used as opposed to using healthy control children, restrictive lung disease would have been over-estimated in SCD.

Finally, given that ethnic differences in lung function have been attributed to differences in anthropometry,[8,9,211] the use of healthy control children proved to be a vital inclusion in the study when describing anthropometry. Despite the careful selection of the anthropometric reference data, important differences in anthropometry would have been missed had a control group not been recruited. Height, weight and BMI Z Score (according to the CDC growth charts[131]) in healthy White children averaged around 0.3Z, whereas the Black children were slightly taller, heavier and had a higher BMI than the White children of the same age (with an average Z Score for all outcomes of 0.75). Although the Black children were "bigger" than White children at any given age, lung volumes (TLC) were smaller compared to their White peers. Since restrictive lung disease is associated with obesity,[212] the reduced lung volumes observed in the healthy Black children in comparison to their White peers could potentially be attributed to the higher BMI in these children, however a difference of 0.5Z Scores in BMI is unlikely to reduce lung volumes to such an extent.[212] A more conceivable explanation is the trunk: leg ratio which was not recorded in the current study but will be evaluated in the future.

A further important advantage of including healthy control children in this study lay in the fact that without a control group the relative growth restriction seen in SCD would not have been detected. The mean height and weight Z Score (according to the CDC growth charts[131]) in the SCD group was ~0.1 Z which would have been considered to be completely "normal" had it not been for the fact that it was on average 0.5 Z Scores lower than the healthy Black children measured. The significant anthropometric differences between SCD and health may in part be the reason for the lung restriction observed in SCD, however, whether the restrictive defect observed was a direct consequence of growth restriction overall or a result of repeated lung injury could not be determined in this study and was beyond the remit of the thesis to investigate small children with SCD with and without a history of repeated acute chest syndrome (ACS) insults.

9.5.2 Impact on future lung function studies

Results from this study have significant clinical implications with regards to the application and interpretation of lung function tests in children. Despite proving to be of little clinical use in the assessment of lung function in SCD, IOS and sR_{aw} methods were thoroughly investigated and guidelines for their application and interpretation in children were improved, particularly with respect to sR_{aw}.[128] In contrast, spirometry and plethysmographic lung volume assessments proved to be vital in the assessment of lung function in SCD and results were consistent with restrictive lung disease. Given the added complexity of performing lung volume measurements, spirometry may be used as a screening measurement, and lung volumes performed when restrictive lung disease is suspected (i.e. reduced FEV_1 and FVC with a normal/elevated FEV_1/FVC).

As yet, there is no evidence as to whether incorporating spirometry and lung volumes into the clinical care of SCD improves patient outcomes, however a number of children assessed in this study have now been referred to a respiratory specialist, and future work will include an audit of interventions and lung function outcomes.

Overall findings from this study highlight the importance of both applying and interpreting results appropriately and ensuring that the choice of lung function test is tailored to the individual disease process as the ability of specific tests to detect abnormalities varies according to the underlying pathophysiology. SCD is characterised by lung restriction,[24] (hence measurements of lung volumes and spirometry were required), and has been associated with obstruction and “asthma-like” symptoms[14] (hence the inclusion of IOS as an assessment of peripheral airway obstruction and sR_{aw} as an effort independent assessment of airways resistance). The interpretative methods and adjustments for ethnicity developed in this thesis can be applied in different disease groups, however given the sample size upon which these recommendations were based results should still be compared to a prospective, ethnically matched control group, and all future studies should include the measurement of sitting height as this potentially more relevant than standing height alone.

In addition to the change in management of the children with SCD participating in this study (i.e. regular lung function assessments with reports to a respiratory specialist), the study has also changed clinical practice at the UCL Institute of Child Health. All children now undergo sitting height measurements and anthropometric results are expressed using the CDC 2000 growth charts.[131] Furthermore the study has raised

awareness regarding both lung function abnormalities in SCD, and the importance of using appropriate reference data for interpreting lung function results in children (see introduction (page 21) for a summary of publications).

9.5.2.1 Summary of lung function tests suggested for monitoring SCD

Measurements of spirometry and lung volumes are essential to identify restrictive lung disease, furthermore measurements of IOS and sR_{aw} may be useful to identify the obstructive lung disease sometimes observed in SCD. Given the technical difficulties associated with performing lung volumes, the lack of reference data for IOS, and the confounding effects restriction may have on sR_{aw} the primary lung function test recommended for monitoring SCD in young children is spirometry. This should be performed at least annually and more frequently if significant symptoms suggest abnormalities. If spirometry indicates a restrictive pattern (i.e. reduced FEV_1 and FVC but normal FEV_1/FVC ratio) plethysmographic lung volumes should be considered. If spirometry identifies an obstructive pattern (i.e. reduced FEV_1 and/or reduced FEV_1/FVC) bronchodilator responsiveness should be assessed. In addition to measurements of spirometry, assessments of Transfer factor may also elucidate additional important information regarding the pathophysiology of SCD in children old enough to complete such measurements satisfactorily.

9.6 Future research directions

The following areas of research are either currently being undertaken or will be addressed in the future:

9.6.1 Longitudinal studies of SCD

The children with SCD assessed in this study were recruited as part of the SAC study and analysis was limited to cross-sectional data only. The SAC study is funded until 2015 and will continue to take annual measurements of lung function (spirometry and plethysmography) in all children included in this study as well as healthy control children. Measurements of sitting height and respiratory questionnaires will also be undertaken in all of these children. In addition funding will be sought and the ethics committee will be contacted to include the measurement of diffusion capacity of the lung for carbon monoxide (DL_{CO}). This should explain the underlying pathophysiology of the disease and determine the impact of restrictive growth on lung function.

9.6.2 Assessment of anthropometry in health

Many of the healthy children included in this thesis were recruited as part of the SLIC study. This study has funding until 2014 and is currently undertaking detailed measurements of body shape, size and composition in 1600 primary school children of all ethnicities. Assessments will be repeated after one year to determine the extent to which the known variability in lung function between children of different ethnic groups is explained by differences in body physique (size, shape and composition) (www.ucl.ac.uk/slic).

9.6.3 Global lungs Initiative

The Global Lungs Initiative (GLI) aims to develop improved 'All-Age' lung function reference equations across several ethnic groups and to recommend a global approach to the interpretation of spirometry data (www.lungfunction.org). A manuscript describing the new GLI 2012 equations has been submitted to the ERS and future work will involve re-analysing the spirometry data within this thesis using the new Global All-Age reference data to assess the extent to which the new GLI equations are appropriate for Black children.

Phase 2b of the GLI taskforce remit involved re-evaluating QC criteria for children. The analysis of spirometry data from two international centres was completed recently by five expert reviewers including the author of this thesis, on-going work on appropriate QC guidelines for spirometry will continue.

9.6.4 Application for ATS/ERS taskforce status for sR_{aw}

The aim of the taskforce will be to focus on improving the applicability of plethysmographic sRaw measurements in both clinical practice and research, including collecting evidence-based information to underpin standardisation of data collection and analysis. This taskforce will be lead by Jane Kirkby and Dr Paul Robinson

9.6.5 Liaison with manufacturers to improve lung function equipment

As a result of the findings in this thesis and previous studies, communication with the manufacturers of lung function equipment has commenced: Software issues surrounding data extraction and reporting of IOS data have impeded the progress of this technique. Despite limitations surrounding the reference equations for IOS, there are currently no published IOS reference data incorporated into the Jaeger V4.65 device. Communication with the manufacturers has resulted in an agreement that reference equations will be improved in newer software versions. Similarly with sR_{aw}, software modifications to enable accurate measures of flow and implementation of the reference equations[128] need to be achieved to improve the use of this technique in children.

In the field of spirometry, the implications of using inappropriate reference equations was highlighted in a recent letter to the ERJ by the author of this thesis.[154] There has been some delay in ensuring the All-Age[126] reference equations are implemented into the software but progress is being made and a current list of the manufacturers which have implemented these equations can be found at www.lungfunction.org. Further work will need to be done to ensure the new 2012 equations are incorporated into the spirometers once they become available.

9.6.6 Extend the measurements to older children/other disease groups

Finally, the study will be extended to include older healthy children and children with SCD to elucidate the impact of puberty on these important measurements. Measurements in both healthy White and Black children of all ages and ethnicities are required to enable these measurements to be successfully applied and interpreted in other disease groups.

9.7 Conclusions

The work presented in this thesis has advanced the field in the application and interpretation of lung function measurements in children. Comprehensive guidelines and reference data for sR_{aw} have been developed and published.[128] Furthermore the extent of the ethnic differences occurring in plethysmographic lung volumes and spirometry was identified and interpretative recommendations were developed.[146] Despite developing recommendations for IOS the use in the clinical monitoring of lung function in SCD was limited, and sR_{aw} failed to differentiate children with SCD from healthy children. Spirometry and plethysmographic lung volumes however, proved to be extremely useful in differentiating lung function in children with SCD from health and demonstrated a restrictive lung disease pattern in the children with SCD assessed. Despite the high prevalence of respiratory symptoms, and the suggestion of airway obstruction in some studies, this thesis demonstrated that asthma is not a common feature of SCD. Furthermore, the new interpretation strategies developed in this thesis will facilitate better interpretation of lung function in Black children.

To date, this is the only study which has investigated the application and interpretation of IOS, sR_{aw}, spirometry and lung volumes in healthy Black and White children aged 4 to 12 years. Moreover, no other study has applied all four lung assessments in SCD. The study highlighted the importance of including an appropriate control group since the extent of both growth restriction and the pattern of restrictive lung disease in SCD would not have been detected had comparisons been made directly to published reference data. Results from this study therefore improve the overall standards for using these lung function tests in children. Furthermore, results from this study may be transferred to different settings (i.e. additional laboratories, schools, and other field settings) and can be used to inform on both power calculations and suitable outcome measures for clinical trials/observation studies.

A unique cohort of children with SCD with ethnically matched control children has been established as result of this thesis and, with continued funding and improvements to the applications and interpretation of lung function tests in children, respiratory management of children with SCD and other lung diseases will hopefully be enhanced.

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Appendix 1: Publications

Appendix 2: Poster presentations

Appendix 3: Pilot studies

Specific airways resistance: The impact of bacterial filters.

Two centres routinely used bacterial filters, and one only used filters for specific patient groups (e.g. cystic fibrosis). To establish the impact of using bacterial filters, paired sets of sR_{aw} measurements were obtained in 9 adults using a mouthpiece and noseclip with and without a bacterial filter *in situ* at a breathing frequency of 30bpm within a 10 minute interval. The equipment was calibrated with a filter *in situ* and the "filter check box" within the software was checked for all measurements i.e. the software had an internal adjustment that corrected for the added resistance created by the filter at all times, therefore the absolute difference of not having a filter *in situ* could be measured. The measurements were not taken in a random order as infection control policies meant that the pneumotach had to be disinfected after measurements which did not include a filter *in situ*. All measurements were obtained by the same operator and analysed by a different operator who was masked to the measurement condition. Paired t-tests were used to determine the differences with and without filters.

Results: sR_{tot} was significantly higher when filters were used; mean difference (95%CI): 0.19kPa·s (0.13; 0.25) (Figure A).

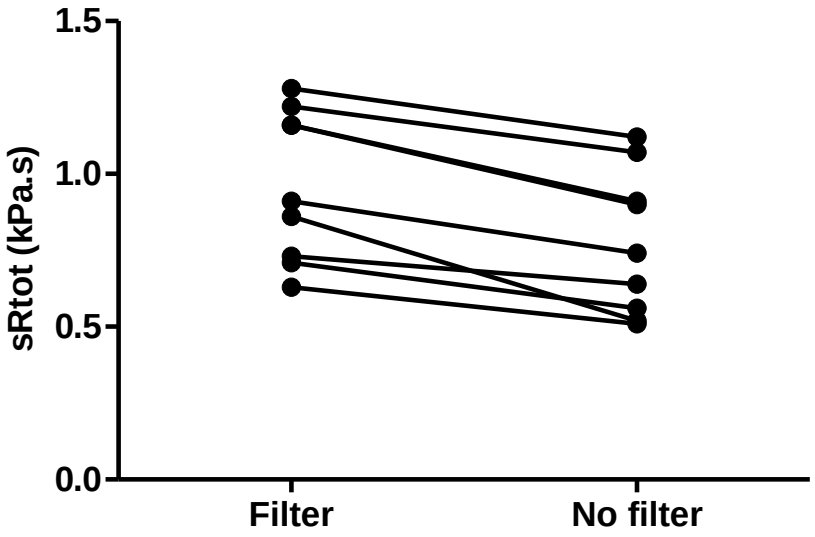

Figure A: Comparison of sR_{tot} when recorded with or without a bacterial filter.

Appendix 4: Patient information sheets

- SAC parent invitation letter
- SAC parent information leaflet
- SAC child information leaflet

- SLIC parent invitation letter
- SLIC parent information leaflet
- SLIC child information leaflet

Appendix 5: Consent forms

- SAC consent form
- SLIC consent form

Appendix 6: Respiratory questionnaire

- SAC health questionnaire
- SAC screening questionnaire for healthy controls
- SLIC health questionnaire