

Reference Equations for Specific Airway Resistance in Children:

The Asthma UK Initiative

SHORT TITLE: Specific Airway Resistance in Young Children

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ABSTRACT:

Introduction: Plethysmographic Specific Airway Resistance (sR_{aw}) is a useful research method for discriminating lung disease in young children. Its use in clinical management has, however, been limited by lack of consensus regarding equipment, methodology and reference data.

Aims: To collate reference data from healthy children (3-10y), document methodological differences, explore the impact of these differences and construct reference equations from the collated dataset.

Methods: Centres were approached to contribute sR_{aw} data as part of the AsthmaUK initiative. A random selection of pressure-flow plots were assessed for quality and site visits elucidated data collection and analysis protocols.

Results: Five centres contributed 2,872 measurements. Marked variation in methodology and analysis excluded two centres. sR_{aw} over-read sheets were developed for quality control. Reference equations and recommendations for recording and reporting both specific effective and total airway resistance (sR_{eff} and sR_{tot} respectively) were developed for White European children from 1908 measurements made under similar conditions.

Conclusions: Reference sR_{aw} data collected from a single centre may be misleading, as methodological differences exist between centres. These preliminary reference equations can only be applied under similar measurement conditions. Given the potential clinical usefulness of sR_{aw} , particularly with respect to sR_{eff} , methodological guidelines need to be established and used in prospective data collection.

KEYWORDS: Airway Resistance; Children; Lung function; Plethysmography; Preschool children; Reference values.

INTRODUCTION:

Lung function techniques that can be applied during tidal breathing are particularly pertinent in young children where active cooperation and understanding may be limited[1]. Plethysmographic Specific Airways Resistance (sR_{aw}) can be measured during tidal breathing from the relationship between simultaneous measurements of airflow and change of plethysmographic pressure without need for any special breathing manoeuvres against an airway occlusion[2] and is therefore ideally suited for young children[3-6]. sR_{aw} is the product of Functional Residual Capacity (FRC) and Airways Resistance (R_{aw}). Since R_{aw} has a strong inverse relationship to lung volume[2], theoretically sR_{aw} should provide a relatively stable index with which to distinguish effects of disease from those of growth and development. There is, however, some evidence to suggest age and/or gender differences in young children[7, 8]. This technique has proved to be a feasible and useful outcome measure in clinical research studies of preschool children with cystic fibrosis and wheezing disorders[9-15].

Despite these advantages, the use of sR_{aw} 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. Many users have therefore developed their own in-house techniques for data collection, analysis and quality control. Consequently, reported values of sR_{aw} have been collected under a variety of differing measurement conditions involving, including modified masks[15] or mouthpieces[16]; use bacterial filters or not and different breathing patterns and frequencies.

Results are further influenced by:

- a) the extent to which operator quality control 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[11] or the weighted mean of data selected after extensive quality control[12].
- d) which outcomes are used: i.e. ‘effective resistance’ (sR_{eff}); ‘total resistance’ (sR_{tot}); ‘peak resistance’ (sR_{peak}) or that calculated over a fixed range of flow (e.g. between 0-0.5L/s i.e. $sR_{0.5}$).

In children, the most common reported outcomes are sR_{eff} and sR_{tot} ; $sR_{0.5}$ has been discouraged in children due to potential age-related effects[17]. sR_{tot} is a simple outcome measured between points of maximum plethysmographic (box) pressure, whereas sR_{eff} is calculated from multiple points throughout the breathing cycle (the integration method, see OLS for details) and may thus be a better reflection of airway mechanics[7, 18].

Interpretation of sR_{aw} is further complicated because commercially available plethysmographs now apply a digital (electronic) ‘thermal correction’ factor during calculation of sR_{aw} [19], whereas default reference equations are commonly based on data collected using the re-breathing or panting technique to achieve body temperature,

pressure and water vapour saturated (BTPS) conditions[20]. Since the latter are known to be systematically lower than those collected under electronic conditions[5], even healthy subjects will appear to have abnormally elevated sR_{aw} if results are interpreted using BTPS-derived reference data.

The Asthma UK Collaborative Initiative was established to collate available reference data from healthy young children to produce reference centiles for Spirometry[21], Respiratory Resistance from the interrupter technique and plethysmographic sR_{aw} (www.growinglungs.org.uk). This study aimed to:

- 1) collate available reference data for sR_{aw} and document any differences between the collaborating centres,
- 2) explore the impact of these differences and
- 3) construct reference equations from the collated dataset.

MATERIALS AND METHODS:

STUDY SUBJECTS: 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.

STUDY DESIGN: sR_{aw} data were collected in healthy children aged 2-11 years, together with details regarding population characteristics, equipment, measurement protocols and quality control. All data were collected using the same plethysmographic body box (Jaeger GmbH, Wurzburg, Germany) though different software versions were used. Where possible, visits were made to collaborating centres to conduct inter-lab comparisons and obtain random samples of original pressure-flow (P/F) curves. All data were anonymised prior to contribution and came from research studies where full local ethics approval and informed parental consent had been obtained. We determined differences in sR_{aw} of 0.2kPa·s between centres or 0.1kPa·s within-subject as being clinically or physiologically significant, such differences approximating one standard deviation (SD) for between and within-subject variability respectively[3].

METHODS: Where differences in methodology between-centres were observed, sub-analyses were conducted to establish the impact of these differences (details of these sub-analyses and results can be found on the OLS).

Quality Control: A random sample of 10-20 P/F curves from children studied at each centre was requested to enable a central quality control (QC) over-read; P/F curves were graded out of 6, with one point given for each of the following criteria achieved:

- 1) Respiratory rate 30-45bpm;
- 2) Breaths super-imposable (i.e. parallel tangents);
- 3) Breaths similar size and shape;
- 4) Breaths reasonably closed at zero flow;
- 5) No obvious distortions (e.g. glottic closure, cough, talking)
- 6) Availability of at least two acceptable trials

The over-read sheet and instructions can be found on the OLS and at www.growinglungs.org.uk

sR_{aw} outcomes: The potential impact of reporting different outcome measures for sR_{aw} was investigated by reanalysing a subset of data and making within-subject comparisons between sR_{tot} and sR_{eff}.

Reporting results: Data with 3 sets of 10 breaths (or 5 sets of 5 breaths, depending on software version) were examined and results from each reported as:

- a) the *weighted mean*, i.e. the sum of all ‘acceptable’ sR_{tot} values, after rigorous QC, divided by the total number of acceptable values[12]. Exclusion of ‘technically unacceptable data’ was based on the QC criteria 1-5 as detailed above.
- b) the *mean of the median* sR_{tot} from three trials, prior to any exclusions; or
- c) the ‘median’, as represented by the median value of sR_{tot} from the most representative (i.e. ‘median’) trial.

ANALYSIS: Statistical analyses were performed using SPSS V16, and Graph-Pad Prism 5. One-way ANOVA and independent t-tests were applied to assess between-centre differences. Paired t-tests and Bland and Altman plots were used to assess within-subject agreement between different outcomes (sR_{eff} and sR_{tot} , and within the same subject over time). Where appropriate, linear regression analyses were used to assess the relationship between different sR_{aw} outcome measures to determine correction factors. Reference equations were developed using the LMS method[22] details of which can be found in the OLS.

RESULTS:

Five centres contributed 2,872 sets of sR_{aw} data from 2,347 children measured between 1995 and 2008. All centres had used a Jaeger Plethysmograph, but five different software versions were used ranging from 4.01 to 5.01 (Table 1). Individual sR_{aw} values ranged from 0.21 - 2.82kPa·s, with the mean (SD) sR_{aw} from these centres ranging from 0.55(0.18) to 1.29(0.30)kPa·s. Significant differences were observed between centres (One-way ANOVA: $p < 0.0001$) (Figure 1).

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. The limited data in non-white subjects precluded analysis according to ethnic origin, hence these subjects were excluded from the reference equations. Further details regarding population characteristics, equipment and methodology are summarised in Table 1.

Table 1: Comparison of population characteristics and details of equipment and methodology used by collaborating centres

Centre	n (%boys)	Age, years Mean(SD) (range)	Height, cm Mean(SD) (range)	Equipment:			Quality control: (computer / manual selection)	sR _{div} outcomes:	Reporting of results
				Software	Mask or mouthpiece	Use of Filter			
1	38 (58%)	7.1 (1.1) (4.8, 8.9)	123.3 (7.8) (109, 141)	V4.1	mouthpiece	In specific patient groups	computer & manual	sR _{tot} & sR _{eff}	Weighted mean
2	40 (50%)	4.5 (1.0) (2.6, 6.0)	107.5 (10.3) (88, 127)	V4.22 & V4.34	Specialised mask	No	computer	sR _{tot}	Mean of Median
3	828 (55%)	5.1 (1.0) (2.5, 7.0)	111.6 (8.1) (88, 155)	V4.34	mouthpiece	No	manual	sR _{tot}	Median of first set of acceptable loops
4	472 (51%)	6.6 (2.1) (2.7, 11.0)	120.3 (14.5) (89, 159)	V4.65 & 5.01	mouthpiece	yes	computer	sR _{tot} & sR _{eff}	Weighted Mean, mean of medians & median
5	1494 (53%)	4.2 (1.0) (3.0, 5.0)	104.3 (8.2) (87, 126)	V4.34 & 4.65	mask	yes	computer	sR _{eff}	Mean of Median

Equipment: Three centres used a mouthpiece and nose clip for data collection while the remaining two used a modified facemask.

Quality Control: All centres supplied details regarding methodology and analysis, and a random sample of original P/F curves for over-reading; however, the print-outs from one centre were too small to over-read and another centre only provided a single screen-shot of recent data. Examination of the protocols revealed two centres (1 and 3) had performed “manual adjustment of the tangent” whereas the others accepted the computer generated slopes. sR_{aw} was significantly lower when manual adjustment was used (Figure 1) and results from these centres (n=866) were excluded from further analysis. The three remaining centres scored 5/6, 3/6 and 5/6 on over-read. (See OLS for details). All subsequent results are based on the three remaining centres.

sR_{aw} Outcomes: In the two centres which reported both sR_{eff} and sR_{tot} the outcomes were highly correlated (Figure 2a) but sR_{eff} was systematically lower (Figure 2b). We used the data from centre 4 to generate a correction factor which was validated with data from centre one (data not shown), we then applied this correction factor to calculate sR_{eff} and sR_{tot} in all centres to allow direct comparisons (Table 2). Values of sR_{eff} and sR_{tot} were similar between the centres, as was the between-subject variability.

Table 2: The mean (SD) sR_{aw} values for all included centres:

Centre	sR_{eff}	sR_{tot}
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) _{##}

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})

Mean vs. Median: Within-subject comparisons revealed no statistical differences between weighted-mean vs. mean-of-median sR_{tot} (mean difference: 0.003 (95%CI - 0.001; 0.006) kPa·s, n=297) or between mean-of-median and median-median sR_{tot} data sets (mean difference: -0.02 (95%CI: -1.90; 0.15) kPa·s, n=101).

Repeated measurements: 525 repeated measurements at 3 and 5 years of age were available from one centre. A very small, albeit statistically significant within-subject reduction in sR_{eff} occurred over this period: mean difference (95%CI) in sR_{eff}: -0.06 (- 0.08; -0.04)kPa·s (p<0.0001), suggesting minimal age-related changes (Figure 3).

Age effects: After adjustment for centre, sex and age were independently associated with sR_{aw}; sR_{eff} decreased with age (β : -0.044, p<0.0001), and was slightly lower in females (β : -0.030, 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_{eff} was independent of height (β : 0.002, p=0.94)

Within-centre differences: In Centre 4, healthy subjects were measured as part of five different projects, one of which was carried out across three different sites. Despite use of identical protocols and equipment during all projects there were statistically significant differences in mean sR_{aw} between projects; these differences being of potential clinical/physiological relevance in two projects (1b and 4), (Table 3).

Table 3: Demographics and sR_{tot} results from projects co-ordinated from centre 4.

Project	Data collection	Software version	n	Age, years	Height, cm	Over-read score	Peak-peak Flow (L/s) [range]	sR _{tot} kPa·s
1a	2000-2003	4.65	32	7.4 (0.7)	127.1 (7.4)	6/6	2.1 [1.2-3.6]	1.3 (0.3)
1b	2000-2003	4.65	31	7.6 (0.6)	127.5 (6.4)	5/6	1.1 [0.6-2.2]	0.9 (0.3)
1c	2000-2003	4.65	58	7.7 (0.6)	127.8 (6.6)	6/6	1.6 [1.0-2.4]	1.1 (0.3)
2	2000-2003	4.65	160	4.6 (1.1)	107.5 (8.5)	4/6	1.6 [1.0-2.3]	1.3 (0.3)
3	2006-2008	4.65 & 5.01	72	7.6 (1.2)	126.2 (9.3)	6/6	1.8 [1.3-2.8]	1.3 (0.3)
4	2006-2008	5.01	70	5.5 (0.8)	112.6 (6.7)	5/6	1.7 [1.5-2.0]	1.0 (0.2)
5	2007-2008	5.01	49	10.4 (0.5)	145.9 (6.6)	6/6	2.1 [1.5-3.2]	1.2 (0.2)

Results summarised as mean (SD) except for flows which are median [range]

Reference Equations: LMS reference equations were developed from the 1908 included measurements (Figure 4). The reference equations were limited to children aged 3-10 years to avoid edge effects. Z-scores can be obtained by substituting the values for M, S and L from Table 4 into the following equation: $z\text{-score} = [(Measurement/M)^L - 1] / [L \times S]$

Table 4: Reference equations for sR_{tot} and sR_{eff} for children aged 3-10years. Age is in decimal years; for sex enter 1 for males and 2 for females; exp (exponentiate).

	M	S	L
sR _{tot}	$1.3083378 - 0.0001648 * \text{age}^3 - 0.0367030 * \text{sex}$	$\exp(-1.72663 - 0.00428 * \text{age}^2)$	0.04787
sR _{eff}	$1.1426155 - 0.0001369 * \text{age}^3 - 0.0337459 * \text{sex}$	$\exp(-1.650597 - 0.003786 * \text{age}^2)$	0.08805

It is important to note that these equations can currently only be applied to White children of European descent aged 3 to 10 years, and only when measurements are made under the following conditions: Equipment should include a Jaeger Plethysmograph (Version 4.01 or above) with a specialised mask or mouthpiece with noseclip, and a filter *in situ*. No adjustments to the computer generated slope should be made and a breathing frequency of 30-45bpm should be adhered to. More details of recommendations for future data collection, including the use of sR_{eff} as the primary outcome measure since this computes pressure and flow signals throughout the breathing cycle, can be found in Box 1 of the OLS.

DISCUSSION:

This study comprises the largest collation of paediatric sR_{aw} data from healthy controls to date, enabling a comprehensive review of the different methodologies. Significant differences in methodology between collaborating centres necessitated exclusions of some sR_{aw} data; but enabled the development of a quality-control over-read sheet and preliminary sex-specific reference equations which also adjust for the minimal age-related changes in sR_{eff} and sR_{tot} . In addition, we present recommendations to facilitate more standardised data collection and analysis in the future.

Clinical Implications:

The observed methodological differences have important implications in both clinical management and research studies, and suggest that reference ranges obtained in one laboratory could lead to significant under or over-estimation of lung disease if transferred to another, unless measurements are performed under identical conditions. Thus the reference equations presented are an interim solution to the problem, which can **only** be applied to populations that have been measured using the same methodology. Nevertheless, these preliminary equations are far more appropriate than those currently available in Jaeger equipment. The “Jaeger-kids” for children aged 4-18years, and “Jaeger” for those >18years are based on data collected under BTPS conditions over 30 years ago[20] and have identical predicted values for sR_{eff} and sR_{tot} , whereas we found sR_{eff} to be significantly lower than sR_{tot} (Figure 2). Furthermore the ‘Jaeger-kids’ predicted values of both sR_{eff} and sR_{tot} of 0.51kPa·s for girls and 0.53kPa·s for boys <18years significantly under-estimate the actual values observed in healthy children in

this study, which were collected using electronic compensation. This would result in serious over-estimation of the degree of airway obstruction in children with lung disease. In addition, whereas we observed a very gradual decline in the predicted values with age (Figure 3), the Jaeger equations suggest that there is a sudden (and physiologically implausible) *increase* in predicted values to 0.96kPa·s for females and 1.18kPa·s for males from 18 years of age onwards. Finally, in contrast to the current Jaeger reference equations, and more recent ‘single-centre’ reference data[3], limits of normality for both males and females in sR_{eff} and sR_{tot} with which to identify abnormality more reliably in individual children, are now provided.

Strengths and Limitations:

The Asthma UK dataset is the largest collection of sR_{aw} data in children; however all collaborating centres used the Jaeger equipment and we cannot generalise our findings to other equipment. Furthermore the impact of software version could not be examined as several centres had updated software since time of data collection. Quality control is an essential aspect of any lung function test, and our in-depth examination of each centre-specific protocol enabled us to develop a QC over-read sheet.

We demonstrated that sR_{aw} can be affected by use of a filter in adults if not calibrated and adjusted for in the internal settings (see OLS). While the measured effect was within the expected 0.1 kPa·L⁻¹·s increase in resistance as reported by manufacturers (Air Safety LTD, Lancashire, UK) this could introduce an important bias to sR_{aw} if an operator neglects to calibrate with a filter in situ, and/or neglects to select the “filter check box” in

the internal settings. These differences may be greater in children because of the relative increase in dead space. Ethically we were unable to evaluate the influence of filter use in children, but since filters should be used to comply with most infection control policies, reference data should be based on measurement conditions which reflect clinical practice. However, when a filter is used it is essential that the plethysmograph is calibrated with a filter *in situ*, and the internal settings are corrected for the additional resistance imposed by the filter.

It has previously been shown that breathing frequency can have a marked impact on measured values of sR_{aw} [5]. In this study collaborating centres adhered to the recommendations of 30-45bpm, and we were unable to systematically evaluate the influence of breathing frequency. The true impact of breathing pattern may relate more to flows attained, which can vary markedly while maintaining identical breathing frequency, than to respiratory rate *per se*. This warrants further investigation into flows attained and breathing frequency in future studies. In the meantime, we would recommend the child is encouraged to breathe as quietly and naturally as possible while maintaining breathing frequencies between 30-45bpm.

We present reference equations for both sR_{eff} and sR_{tot} ; however, sR_{eff} is likely to be the better outcome as it takes into account resistive changes throughout the breathing cycle rather than simply the tangent between points of maximum pressure[18], (the equation for calculating sR_{eff} is included in the OLS). While the difference between sR_{eff} and sR_{tot} was relatively small in health (and assumed to be similar across all centres), differences

in outcomes may be more marked in the presence of airway disease and it is therefore essential to use a consistent approach and not to attempt to predict one outcome from another in children with lung disease. Whilst, the preliminary reference equations presented within are an improvement on current equations, they must be used with caution since they were created with a correction factor on the assumption the relationship between sR_{eff} and sR_{eff} in health across the centres were consistent. They will, however, enable future research and clinical studies to examine which of these outcomes is more appropriate for detecting changes in lung function in young children with respiratory symptoms and disease.

Repeated measurements within the same children are rare but crucial for understanding growth and development of airway function within individuals. The longitudinal data included as part of this study suggested minimal age-related changes in young children. Nevertheless, the 95% limits of agreement indicated that even in healthy children sR_{aw} may vary by up to 0.5kPa·s over a 2 year period, which must be taken into account when interpreting serial results from those with lung disease. It should also be noted that while most studies have concluded that sR_{aw} is relatively consistent in preschool children, and we only noted minor age related changes between 3 and 10 years in this study. There are developmental reasons why this may not be the case during infancy.[8, 23]

Finally, we observed differences within-centres with no apparent explanation. It has recently been suggested that even when using apparently identical equipment and protocols, inter-centre differences in sR_{aw} can result from “hidden” differences in internal

settings within the equipment[24] which are only accessible to equipment engineers. This could have potentially contributed to the within-centre differences observed in the current study. Such differences require thorough investigation by the manufacturers with standardisation of internal settings prior to distribution.

Recommendations:

Based on the data collated from five European centres we have agreed upon recommendations which will facilitate further improvements to the sR_{aw} technique, such that data collection in the future can be combined to develop more robust reference equations. Detailed recommendations are available in Box 1 of the OLS.

1. 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.
2. While we were unable to directly compare results obtained with a modified mask and mouthpiece, previous studies have found no difference between these methods[4]. 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.
3. As software and equipment change, we recommend 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 i.e. breathing quietly at 30-45bpm with a filter in place.

4. We recommend for future studies sR_{eff} to be the primary outcome measure since this calculates sR_{aw} from multiple points throughout the breathing cycle. sR_{eff} can be calculated as follows:

$$sR_{eff} = (P_{amb} * \text{Integral } \Delta V dV) / \text{Integral } V' dV$$

where: P_{amb} is the ambient pressure; Integral $\Delta V dV$ is equivalent to the area enclosed by the specific work of breathing loop, and integral $V' dV$ is equivalent to the area of the flow / volume-loop.

CONCLUSIONS:

Significant methodological differences between centres that perform sR_{aw} measurements have important implications for clinical interpretation of results. Given the potential clinical usefulness of sR_{aw} , there is an urgent need to apply standardised methodology, and to prospectively collect data in healthy children of all ages and ethnicities in order to develop more robust reference equations for children.

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Legends for Figures:

Figure 1: sR_{aw} results by centre: The difference between the highest reported sR_{aw} (centre 2) and the lowest sR_{aw} (centre 1) was 0.74kPa·s

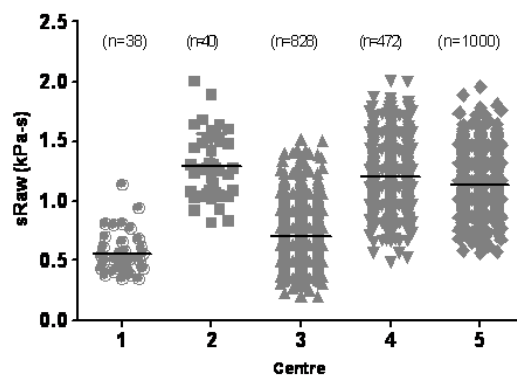


Figure 2: Based on 228 paired measurements sR_{tot} and sR_{eff} were found to be highly correlated ($r^2 = 0.98$; Figure 2a); however, sR_{tot} was significantly and systematically higher than sR_{eff} (mean difference (95%CI): 0.16 (0.15; 0.17) kPa·s; Figure 2b).

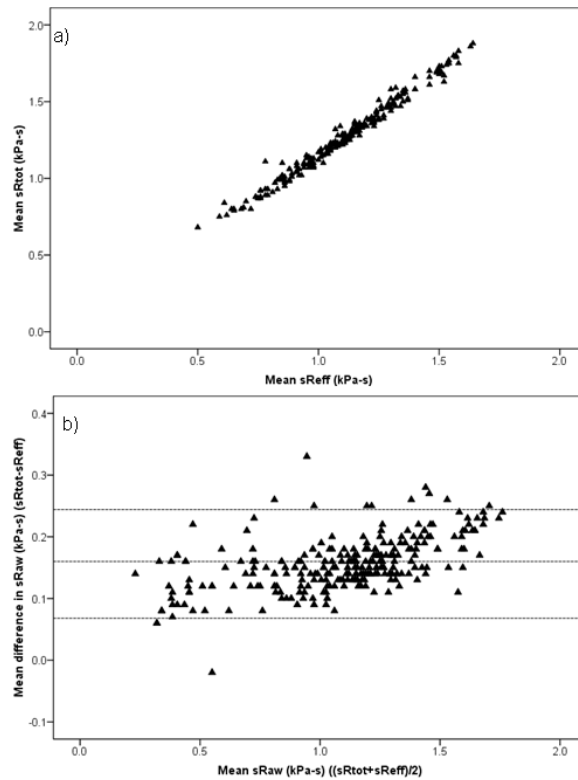


Figure 3: Paired measurements of sR_{eff} at 3 and 5 years of age. Mean difference (95%CI): -0.06 (-0.08 ; 0.04) kPa·s. 95% limits of agreement: -0.54 ; 0.41.

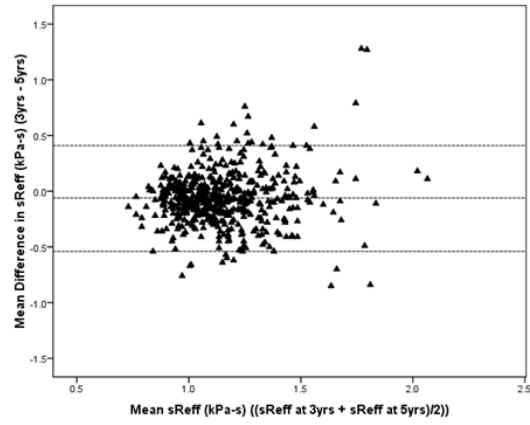


Figure 4: Predicted values of sR_{aw} (kPa·s) with upper and lower limits of normal, (a) sR_{eff} and (b) sR_{tot} for children aged 3-10 years. Solid lines represent equations for boys, whereas dotted lines represent equations for girls.

