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Evaluation of the double-tracer gas single-breath washout test in a pediatric field study

Short title: Double-tracer gas test in a pediatric field study

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Key Words: Adolescent, Child, Helium, Lung Function Tests, Small Airway Remodelling, Sulfur Hexafluoride, Ventilation Tests, Wheezing

Abbreviations

BMI body mass index

DTG-SBW double-tracer gas single-breath washout

FEV_1 forced expiratory volume in the first second

FVC forced vital capacity

FeNO fraction of exhaled nitric oxide

He helium

IQR interquartile ranges

ppb parts per billion

SIII phase III slope

SnIII phase III slope normalized for expired tidal volume

SD standard deviations

SF_6 sulfur-hexafluoride

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IQR interquartile ranges

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SIII phase III slope

SnIII phase III slope normalized for expired tidal volume

SD standard deviations

SF_6 sulfur-hexafluoride

Abstract

Background: The early-life origins of chronic pulmonary diseases are thought to arise in peripheral small airways. Predictors of ventilation inhomogeneity, a proxy of peripheral airway function, are understudied in schoolchildren.

Research Question: Is the double-tracer gas single-breath washout (DTG-SBW) measurement feasible in a pediatric field study setting? What are the predictors of the DTG-SBW derived ventilation inhomogeneity estimate in unselected schoolchildren?

Study Design and Methods: In this prospective cross-sectional field study, a mobile lung function-testing unit visited participating schools in Switzerland. We applied DTG-SBW, fraction of exhaled nitric oxide (FeNO), and spirometry measurements. The DTG-SBW is based on tidal inhalation of helium (He) and sulfur-hexafluoride (SF_6) and the phase III slope ($SIII_{He-SF6}$) is derived. We assessed feasibility, repeatability, and associations of $SIII_{He-SF6}$ with the potential predictors anthropometrics, presence of wheeze (i.e. parental report of ≥ 1 episode of wheeze in the prior year), FeNO, forced expiratory volume in the first second (FEV_1), and FEV_1/forced vital capacity (FVC).

Results: In 1782 children, 5223 DTG-SBW trials were obtained. The DTG-SBW was acceptable in 1449 (81.3%) children, coefficient of variation was 39.8%. $SIII_{He-SF6}$ was independently but weakly positively associated with age and BMI. In 276 (21.2%) children, wheeze was reported. $SIII_{He-SF6}$ was higher by 0.049 $g.mol.L^{-1}$ in children with wheeze as compared to those without and remained associated with wheeze after adjusting for age and BMI in a multi-variable linear regression model. $SIII_{He-SF6}$ was not associated with FeNO, FEV_1, and FEV_1/FVC.

Interpretation: The DTG-SBW is feasible in a pediatric field study setting. On the population level, age, body composition and wheeze are independent predictors of peripheral airway function in unselected schoolchildren. The variation of the DTG-SBW possibly constrains its current applicability on the individual level.

Clinical Trial Registration: ClinicalTrials.gov. NCT03659838

The early-life origins of respiratory diseases such as chronic obstructive pulmonary disease are thought to arise in small airways of lung periphery[1]. Due to practical constrains, predictors of peripheral airway function, *i.e*. ventilation inhomogeneity, remain understudied in large pediatric populations. The double-tracer gas single-breath washout (DTG-SBW) test may overcome these constraints. The DTG-SBW is a simple lung function test based on tidal in- and exhalation of Helium (He) and sulfur-hexafluoride (SF_6)[2,3]. The derived slope of phase III ($SIII_{He\text{-}SF6}$) measures ventilation inhomogeneity of He and SF_6 which differ in diffusive gas mixing properties in small airway compartments[2,3]. The $SIII_{He\text{-}SF6}$ measurement is reliable in research settings and captures altered ventilation inhomogeneity in children with asthma or cystic fibrosis[2-6].

DTG-SBW may be a simple and accessible tool to allow for early detection of lung function alterations, i.e. ventilation inhomogeneity, associated with negative respiratory disease outcomes. However, in unselected pediatric populations, feasibility and repeatability of the DTG-SBW, and predictors of the $SIII_{He\text{-}SF6}$ are unknown. Possible predictors of ventilation inhomogeneity constitute age, sex, body composition, wheeze, airflow limitation and airway inflammation[7-9]. Previous studies suggest that high body mass index (BMI) is associated with dysanaptic lung growth, a non-proportional growth of the airways and lung, as adipose tissue and pro-inflammatory mediators affect lung growth and development. Pediatric wheeze and airflow limitation increase the risk of chronic obstructive pulmonary disease in adults[10].

The current study addressed two research questions: Is the DTG-SBW measurement feasible in a pediatric field study setting? What are the predictors of the DTG-SBW derived ventilation inhomogeneity estimate in a sample of school children? To accomplish this, we applied the DTG-SBW test in a large pediatric field study to assess its feasibility and reliability, and explore associations between $SIII_{He\text{-}SF6}$ and anthropometric variables, wheeze, and standard lung function indices.

Previous estimates of feasibility and intra-test variability of the SBW test in children and adults ranged from 74-89% and 13-24%, respectively[11,12]. For multiple breath washout, the success rates ranged between 50-100% in children[13-15]. We hypothesized that the feasibility and intra-test

variability of the DTG-SBW applied in unselected schoolchildren in a field study setting were >75% and <25%, respectively.

We further hypothesized that the $SIII_{He\text{-}SF6}$ is associated with age and body composition[7], wheeze[9,16], spirometry indices, and fraction of exhaled nitric oxide (FeNO).

STUDY DESIGN AND METHODS

LuftiBus in the School (LUIS) is a prospective cross-sectional observational field study in unselected school-aged children (ClinicalTrials.gov: NCT03659838)[17]. Inclusion criteria were age six to 17 years, German language skills, and consent to participate. There were no predefined exclusion criteria. A mobile lung function-testing unit (motorbus) visited 37 schools in the canton of Zurich, the most populated canton in Switzerland, between 2013 and 2016 [17]. Most children were born in Switzerland (88%) and predominantly of white European ancestry (75.8%). The distribution of the Swiss socioeconomic position index (Swiss-SEP) for families participating in the study was representative to the Swiss-SEP distribution from families with at least one child living in the household from the canton of Zurich[17]. LUIS took place throughout different seasons (e-Figure 1). A consecutively recruited convenience sample of the whole population was studied, as the hardware for DTG-SBW including tracer gas supply became available later during the study. Details about study design, sample size estimates, and data collection have been described recently[17]. Children performed lung function tests in the following sequence: DTG-SBW, FeNO measurement, spirometry. The ethics committee of the canton of Zurich approved the study (KEK-ZH-Nr: 2014-0491). Parents or caregivers signed the informed consent form. Children assented verbally and those aged ≥15 years also signed the informed consent form.

Anthropometrics were measured in the bus on site and parental questionnaires were used to collect information on exposures, respiratory symptoms, diagnoses and prescribed medication[17]. Wheeze was specified as parental report of continuous whistling sound during expiration during one or more episodes in the past 12 months[17].

Tidal DTG-SBW was performed in triplet using the Exhalyzer D® (EcoMedics AG, Duernten, Switzerland) according to recommendations[18]. An inert double-tracer gas mixture containing 5% SF_6, 26.3% He, 21% oxygen and balance nitrogen was inhaled during a single tidal breath and tidally exhaled to functional residual capacity. The setup, protocol and quality control criteria were in accordance with the *European Respiratory Society* consensus on inert gas washout testing and were previously described[3,17,18]. The DTG-SBW was analyzed automatically followed by quality control using a customized software platform (LungSim based on Matlab® (R2014a, The Mathworks Inc. Natick, MA, USA)[17]. Quality control was performed by two trained lung function technicians and included central over-read. The DTG-SBW trials were categorized according to the quality control (qc) categories A, B or failed (F). The quality control protocol used can be found in the online supplement (e-Table 1). Only children who achieved at least two acceptable DTG-SBW trials were included.

The primary outcome measure was the mean $SIII_{He\text{-}SF6}$ of all technically acceptable DTG-SBW curves of each subject. The $SIII_{He\text{-}SF6}$ was computed from the volumetric expirogram by fitting a linear regression slope to the molar mass signal between 65 and 95 % of the expired volume. In addition, the $SIII_{He\text{-}SF6}$ was normalized for expired volume by multiplication with the expired tidal volume ($SnIII_{He\text{-}SF6}$) as a secondary outcome[17]. Findings are reported in the online supplement. Both lower as well as higher $SIII_{He\text{-}SF6}$ values as compared to a healthy reference population have been shown to be associated with ventilation inhomogeneity arising in central or peripheral airways, respectively[2-6].

Fraction of exhaled nitric oxide (FeNO, parts per billion, ppb) was measured according to recommendations using a single-breath online method and a fast response chemiluminescence analyzer (CLD 88, EcoMedics AG)[19]. Further details on test performance and quality control were previously described[17]. The FeNO is a proxy of eosinophilic airway inflammation, FeNO values ≥ 20 ppb can be considered elevated[20].

Spirometry was performed using a standard spirometer (Masterlab, Jaeger, Wuerzburg, Germany) according to recommendations[21]. Indices were forced expiratory volume in the first second (FEV_1), and the ratio of FEV_1 over the forced vital capacity (FEV_1/FVC). Values were expressed as z-score according to *Global Lung Initiative* reference equations[17,22]. Lower limit of normal of FEV_1 and FEV_1/FVC were set at ≤ -1.645 z-score as recommended[21,22].

Analysis

Discrete variables were expressed as counts (percentages) and continuous variables as mean (standard deviation (SD)) or median [interquartile range, IQR], as appropriate. Missing data were not imputed[17]. Between group differences were assessed using unpaired t-tests for parametric and Wilcoxon-Mann-Whitney-Test for nonparametric estimates. DTG-SBW test feasibility was determined as the success rate calculated as the percentage of children with at least two acceptable trials of all children attempting the test. Intra-test repeatability was calculated as coefficient of variation. The success rate of DTG-SBW was calculated as the number of successful DTG-SBW trials as a percentage of all DTG-SBW trials performed per subject.

Associations were assessed using scatterplots, Pearson's correlations, and univariable linear regression models. Potential predictors of $SIII_{He\text{-}SF6}$ included age, sex, height, weight and BMI z-score, wheeze, and FeNO, FEV_1 and FEV_1/FVC. A multivariable linear regression model was used to explore these variables as independent predictors of $SIII_{He\text{-}SF6}$. Variables were analyzed as continuous variables with their original scale, wheeze as a binary variable (*i.e.* yes or no), and FeNO as quintiles ensuring balanced observations per category. Regression model diagnostics were used to confirm underlying assumptions. P-values < 0.05 were considered statistically significant. All analyses were performed using STATA (StataCorp LP, College Station, TX, USA Version 16.0). Figures were made using GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA).

RESULTS

In total, 3870 children were enrolled into the LUIS study. The children`s median [IQR] age was 12.1 [9.3-14.0] years and half of the population was female. The DTG-SBW test was applied in 1782 (46.0%) children, which were slightly younger (0.7 years), had slightly lower Swiss-SEP (1.3 points), reported hay fever somewhat more frequently (2.7%), and FeNO was slightly lower (2.6 ppb) compared to children not invited to perform the DTG-SBW. There were no systematic differences in anthropometric and lung function estimates between these children (e-Table 2). Anthropometric characteristics and lung function estimates can be found in Table 1 and e-Table 2.

Feasibility and Repeatability

In total, 5223 DTG-SBW trials were obtained of which 4090 trials (78.3%) were of acceptable quality. Thus, 1449 (81.3%) out of 1782 children successfully achieved DTG-SBW tests (e-Tables 3-5). DTG-SBW success rate was higher than the hypothesized success rate (75%). Children with successful DTG-SBW tests were 1.1 years older, had a lower Swiss-SEP, reported wheeze more often than the children with unsuccessful tests, all other anthropometric and questionnaire data were comparable (e-Table 4).

In children with a successful DTG-SBW test, trial quality was rated higher more often. Frequency of higher trial quality control categories was associated with the number of acceptable trials (e-Table 7, 8, and e-Figure 2) until a maximum of 4 trials. The mean (SD) $SIII_{He\text{-}SF6}$ was -0.30 (0.42) $g.mol.L^{-1}$. The repeatability of $SIII_{He\text{-}SF6}$ with a median [IQR] intra-test coefficient of variation of 39.8 [22.0-70.9]% was poorer than the hypothesized repeatability (25%). For more details on DTG-SBW feasibility and repeatability we refer to the online supplement (e-Figure 3, e-Table 9).

Predictors of Ventilation Inhomogeneity

The $SIII_{He-SF6}$ was associated with all preselected anthropometric variables except for sex. In univariable regression models, $SIII_{He-SF6}$ was positively associated with age, height, weight, and BMI z-score (Table 2, Figure 2). In a multivariable regression model, only age and BMI remained independent predictors of $SIII_{He-SF6,}$ increasing $SIII_{He-SF6}$ by 0.013 $g.mol.L^{-1}$ per one year increase in age and by 0.060 $g.mol.L^{-1}$ per one z-score increase in BMI, respectively.

In total, 276 children reported wheeze, 1025 children had no wheeze, and 148 children had missing information regarding wheeze and were excluded from this analysis (Figure 1). Children with wheeze were slightly older (0.7 years), heavier (BMI, 0.2 z-score), and reported atopic diseases more frequently (e-Table 10).

FeNO was slightly higher (4.3 ppb), and spirometry lower (FEV_1, 0.21 z-score) than in children without wheeze (e-Table 10). The $SIII_{He-SF6}$ was associated with wheeze in univariable regression models, and remained weakly positively associated with wheeze after adjustment for age and BMI z-score (Table 2). $SIII_{He-SF6}$ was higher by 0.049 $g.mol.L^{-1}$ in children with wheeze as compared to those without), but was not associated with FeNO or with the spirometry indices $FEV_{1,}$ and FEV_1/FVC (Table 2, e-Table 11). A *post hoc* analysis in a sub-group of children with a BMI z-score >1.0 showed similar results as compared to the primary analysis in the whole cohort (e-Table 12).

DISCUSSION

In this large pediatric field study setting, we found that the DTG-SBW measurement was feasible in a mobile bus lung function laboratory. Repeatability was poorer than hypothesized. We identified predictors of ventilation inhomogeneity in unselected schoolchildren. $SIII_{He-SF6}$ was weakly positively associated with age, BMI and wheeze but not with FeNO or spirometry indices. On the population level in sufficiently large samples such as ours, the $SIII_{He-SF6}$ captures a subtle signal of alterations in ventilation inhomogeneity suggesting small airways dysfunction in children with wheeze. However, on the individual level, the $SIII_{He-SF6}$ does not seem sensitive enough to screen for alterations in ventilation inhomogeneity in unselected children.

Interpretation

In this field study, we found an acceptable success rate in unselected schoolchildren. The current success rate was higher than hypothesized (75%) but lower than previously reported (92%) in selected children within research laboratory settings[2]. Due to the field study conditions with possibly more distracting environment compared to standard laboratories and children naïve to the use of sealed mouthpieces, success rates were somewhat lower. This is supported by the observed learning effect during testing in the current study. Previously reported success rates of other tidal breathing protocols were similar compared to our findings[23]. In our study, the reasons for DTG-SBW test failures were mainly variable breathing pattern. Due to time constraints, details of test failure were not recorded on site. In a previous study performed in a lung function laboratory, variable breathing pattern accounted for 94% of DTG-SBW test failures in school-aged children[2]. In that study, reasons for DTG-SBW test rejection were (i) variable tidal flows and volumes, (ii) small tidal volumes lacking phase III of the expirogram, and (iii) technical errors[2].

The coefficient of variation quantifying intra-test variability of $SIII_{He-SF6}$ was higher than previously reported (19%) for DTG-SBW[2] but comparable to the SIII indices Scond and Sacin from the established multiple-breath washout test supporting the reliability of the current analysis[6,24]. The estimated mean value of $SIII_{He-SF6}$ was close to zero in our study, therefore small changes may have increased the coefficient of variation exponentially. The variability seen can be due to factors related to the field-study setting, but estimation of the proportion of variability that can be attributed to the setting, is challenging. It is well established, however, that the intra-test variability for inert gas analysis is high, commonly thought to be due to effects of breathing. Interestingly, variability of $SIII_{He-SF6}$ was associated with age and the variability in tidal volume in our study, but not with other potential explanatory variables, such as the $SIII_{He-SF6}$ value itself. These data suggest that SIII indices are prone to considerable inherent physiological variability, and tidal breathing. Normalization for tidal volume alone may not substantially decrease variability or increase sensitivity of the test[22,25,26]. Current protocols for SIII measurement seem to require refinement prior

to clinical routine application. The high intra-test variability may dampen test sensitivity to estimate subtle physiological signals in individuals. Further research is needed to identify potentially modifiable sources of test variability and assess the potential of alternate protocols to reduce intra-test variability of the DTG-SBW.

Additionally, previous data demonstrated that $SIII_{He-SF6}$ correlates with standard estimates of ventilation inhomogeneity[2-6,24]. However, it is unclear whether $SIII_{He-SF6}$ is also a proxy of structural airway disease. While it is established that in Cystic Fibrosis, the lung clearance index correlates with structural airway changes detected in chest computed tomography, there is one negative study for the $SIII_{He-SF6}$[27]. Multiple-breath washout or lung imaging were not obtained in this field study. Yet these estimates would have allowed more in-depth assessment of the diagnostic performance of $SIII_{He-SF6.}$ Our study provides further evidence, that body composition is a predictor of lung function development. Our data are in line with previous findings suggesting age- or height-dependent effects on ventilation inhomogeneity estimates such as lung clearance index from multiple-breath washout[7,28]. Our data further suggest that unfavourable body composition estimated by BMI may modify ventilation inhomogeneity. Reasons remain speculative but may partly relate to airway dysanapsis observed in children with high BMI[28]. Indeed, we have recently shown that the spirometry indices obtained in this cohort did not fit well the reference values from the *Global Lung Function Initiative*[26]. Underestimation of FEV_1 and FVC in the current cohort was partly explained by BMI, though FEV_1/FVC was not affected.

Wheezy symptoms are common and account for considerable burden in pediatric health care. We found altered ventilation inhomogeneity possibly arising in obstructed small airways related to previous wheezy symptoms[2,4-6]. Interestingly, our study suggests that these alterations in ventilation inhomogeneity were independent of airway inflammation or airflow limitation. However, overlap in $SIII_{He-SF6}$ values of children with *vs* without wheeze was considerable. Comparable to other studies, peripheral airway function estimated by current inert gas tests appears largely normal in children with wheeze[29]. Therefore, the difference in $SIII_{He-SF6}$ in children with wheeze was relatively small and adjustment for age and BMI further increased the confidence

intervals. Comparable to $SIII_{He-SF6}$, FeNO, FEV_1 and FEV_1/FVC values were overlapping between children with *vs* without wheeze suggesting overall relatively low pre-test probability (*i.e.* low prevalence) of lung function abnormalities in the current cohort.

Strengths and Limitations

The large sample size is a strength of this prospective study, as it allows conclusive analyses of potential predictors of lung function. Our study allowed for thorough assessment of potential predictors of the $SIII_{He-SF6}$ estimate, including anthropometric and lung function measures. The large sample of unselected schoolchildren supports the generalizability of our findings. Participation of schools was decided by the heads of the schools which may have introduced selection to some extent. Yet, the Swiss-SEP for families participating in the study was representative for the canton of Zurich[11]. As the DTG-SBW test was introduced later in this study, only a subgroup of the LUIS study was invited to perform DTG-SBW. During this study period, the frequency of measurements varied over time. The $SIII_{He-SF6}$ was not influenced by timing of measurements, *i.e.* seasonal effects.

The current protocol determined the sequence of testing to avoid influences from forced breathing manoeuvres during spirometry on $SIII_{He-SF6}$ and FeNO. Tidal inhalation of inert gas during the DTG-SBW unlikely influenced subsequent FeNO or spirometry measurements.

We report wheeze in 19% of our study population, whereas this was 8% for the total LUIS population. In latter study, wheeze was defined as “whistling or panting sound” originating from the chest within the last 12 months. In the current analysis, we expanded the definition of wheeze by adding “whistling or panting sound” originating from the chest in response to triggers such as exercise, respiratory tract infection, cold air and others.

The proportion of variation in $SIII_{He-SF6}$ in this unselected population, that can be explained by wheeze, was low. We acknowledge that questionnaire-based classification of wheeze may have been subject to recall and misclassification bias. Parent reported wheeze may have been less precise compared to physician reported wheeze. The sound of wheezing that parents notice unaided by a stethoscope (i.e. "audible" wheeze) originates from trachea and larger bronchi, rather

than from the peripheral small airways. We assume that misclassification rather led to underestimation of the strength of association between wheeze and $SIII_{He-SF6.}$ Premature birth may affect lung development and alter ventilation inhomogeneity in some children. We were unable to explore possible effects of prematurity on $SIII_{He-SF6}$.

Outlook

Our results suggest that DTG-SBW is feasible in children between six and 17 years of age. Data from younger children are scant and warrant further study [2,4,30]. Despite of good feasibility, the high variability and presumably low sensitivity to capture slightly increased ventilation inhomogeneity constrain its use in unselected individuals. Currently, the DTG-SBW is applicable in research settings and sufficiently large populations or in selected individuals with high pre-test probability of lung function abnormalities. In the latter, we have shown that the $SIII_{He-SF6}$ is responsive to bronchodilator inhalation in asthma or chest physiotherapy in cystic fibrosis[2-6]. Distinct interpretation of dynamics in $SIII_{He-SF6}$ warrants further research. Future longitudinal studies are warranted to establish the minimal clinically important difference derived from variability estimates and patient reported outcomes.

To conclude, the DTG-SBW measurement is feasible in pediatric field studies. However, relatively high variability of $SIII_{He-SF6}$ appears to limit the interpretation. This makes DTG-SBW currently unsuitable in small populations with low pre-test probability of impaired lung function. In the current relatively large population of unselected schoolchildren, age, body composition and wheeze were identified as predictors of ventilation inhomogeneity estimated by the $SIII_{He-SF6}$. Schoolchildren with wheeze may have alterations in ventilation inhomogeneity which can be attributed to peripheral airway dysfunction.

Take-Home Points

Study question: In a large pediatric field study of unselected schoolchildren, what are the success rate and test variation of the double-tracer gas single-breath washout (DTG-SBW) measurement and what are the predictors of ventilation inhomogeneity estimated by the DTG-SBW?

Results: We found an acceptable success rate, substantial test variation and identified age, body composition and wheeze as independent but relatively weak predictors of ventilation inhomogeneity.

Interpretation: The test variation currently constrains the use of the DTG-SBW in children. However, the current data suggest that schoolchildren with wheeze have alterations in ventilation inhomogeneity which can be attributed to peripheral airway dysfunction.

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358

Table 1 Characteristics of children participating in the LUIS study and invited for DTG-SBW

Participants	**In the LUIS study**	**Invited for DTG-SBW**	**With acceptable DTG-SBW data**
Subjects, n	3870	1782	1449
General characteristics			
Males [%]	1937 [50.1]	889 [49.9]	719 [49.6]
Age (years)	12.1 (2.7)	11.7 (2.8)	11.9 (2.7)
BMI (z-score)	0.1 (1.2)	0.1 (1.1)	0.1 (1.1)
White ethnicity [%]	2933 [75.8]	1349 [75.7]	1107 [76.4]
Swiss-SEP (IQR)	69.5 (62.1-75.9)	69.5 (62.1-75.9)	69.4 (62.1-75.0)
Wheeze, n [%]	735 [19.0]	322 [18.1]	276 [19.1]
Hay fever, n [%]	767 [19.8]	326 [18.3]	277 [19.1]
Atopic dermatitis, n [%]	401 [10.4]	188 [10.6]	160 [11.0]
Asthma diagnosis, n [%]	293 [7.6]	135 [7.6]	115 [7.9]
Asthma medication, n [%]	577 [14.9]	262 [14.7]	218 [15.0]
Lung function			
FeNO (ppb), median (IQR)	12.3 (7.2–21.5)	11.0 (6.3-19.6)	11.1 (6.1-19.7)
FEV_1 (z-score)	-0.5 (1.0)	-0.52 (0.97)	-0.54 (0.97)
FEV_1/FVC (z-score)	-0.2 (1.1)	-0.25 (1.04)	-0.24 (1.06)
$SIII_{He-SF6}$ ($g.mol.L^{-1}$)		-0.30 (0.54)	-0.30 (0.42)

Data are presented as mean (SD) or percentage [%], unless indicated otherwise. All questionnaire data were parent reported. Asthma medication included any inhaled corticosteroids or short-acting or long-acting beta-agonists or systemic treatment such as leukotriene receptor antagonists. DTG-SBW: double-tracer gas (helium sulfur-hexafluoride) single-breath washout, BMI: body mass index. Swiss SEP: socioeconomic position in Switzerland. FeNO: fraction of exhaled nitric oxide, FEV_1: forced expired volume in the first second, FVC: forced vital capacity, $SIII_{He-SF6:}$ DTG-SBW slope of phase III.

359

Table 2 Non-adjusted and adjusted association between $SIII_{He\text{-}SF6}$ and potential predictors

Predictors	Regression coefficients	95% CI	P-value*
Anthropometrics			
Sex (male vs female)	-0.011	-0.050 to 0.028	0.592
Age (year)	0.017	0.010 to 0.024	<0.001*
Height (cm)	0.004	0.003 to 0.005	<0.001*
Weight (kg)	0.005	0.004 to 0.006	<0.001*
BMI (z-score)	0.067	0.053 to 0.086	<0.001*
Symptoms			
wheeze vs no wheeze	0.072	0.024 to 0.120	0.003*
wheeze vs no wheeze, adjusted	0.049	0.002 to 0.096	0.042
Lung function			
FeNO (quintiles)	0.004	-0.010 to 0.018	0.557
FEV_1 (z-score)	0.012	-0.008 to 0.032	0.255
FEV_1/FVC (z-score)	0.005	-0.013 to 0.023	0.606

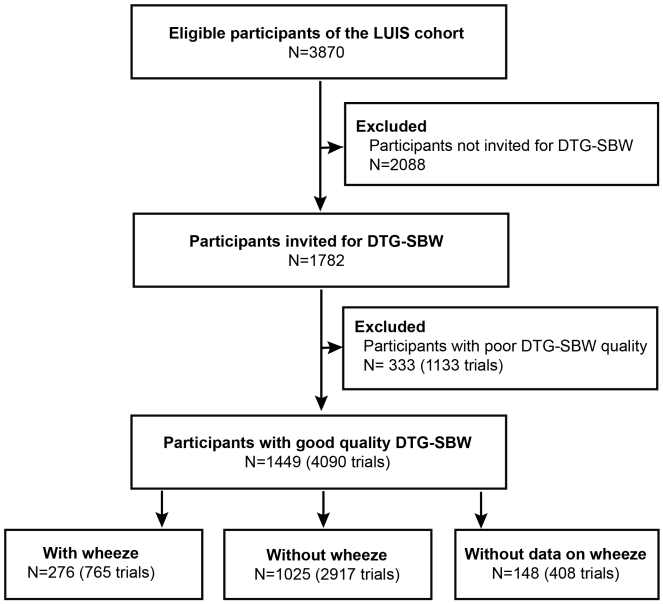
Associations between $SIII_{He\text{-}SF6}$ and potential predictors were assessed using uni- and multivariable linear regression models. Predictors were age, sex, height, weight and BMI; wheeze, FeNO, FEV_1 and FEV_1/FVC. Wheeze was included as a binary variable (i.e. yes or no) and FeNO as data-driven quintiles ensuring balanced observations per category, all other variables were included as continuous variables with their original scale. The quintile boundaries for FeNO were: 0.0-4.9, 5.0-8.8, 8.9-13.8, 13.9-23.4, and 23.5-197.0 ppb, respectively. A multivariable linear regression model was used to assess which anthropometric variables were independent predictors of $SIII_{He\text{-}SF6}$, and the independent predictors age and BMI were used to adjust the association of $SIII_{He\text{-}SF6}$ with wheeze. All associations described the change in $SIII_{He\text{-}SF6}$ in $g.mol.L^{-1}$ induced by one unit increase in the predictor. CI: confidence interval, *: statistically significant difference (<0.05), DTG-SBW: double-tracer (helium sulfur-hexafluoride) gas single breath washout, $SIII_{He\text{-}SF6}$: slope of phase III, $SnIII_{He\text{-}SF6}$: normalized $SIII_{He\text{-}SF6}$, BMI: body mass index (z-score). FeNO: fraction of exhaled nitric oxide, FEV_1: forced expired volume in the first second, FVC: forced vital capacity.

360

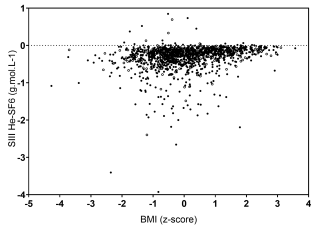
Figure 1 Flow chart of study participants and success rate of DTG-SBW. Out of the 3870 children of the LUIS study, 1782 children performed DTG-SBW (46%). Of these children, 1449 children had acceptable DTG-SBW data (81%). LUIS study: LuftiBus in the school study, DTG-SBW: Double-tracer gas (helium sulfur-hexafluoride) single-breath washout, N: number of children.

Figure 2 Scatterplot of the double-tracer gas (helium sulfur-hexafluoride) single-breath washout derived phase III slope ($SIII_{He\text{-}SF6}$) vs. body mass index (BMI, left panel a) and forced expiratory volume in the first second (FEV1, right panel b). BMI and FEV1 are expressed as z-score. The closed circles display $SIII_{He\text{-}SF6}$ values of children without wheeze and open circles values of children with wheeze. We have excluded one outlier (BMI =-1.7 z-score and $SIII_{He\text{-}SF6}$ = 2.9 g.mol.L-1) in figure panel a) to ease visualization.

373



a)



b)