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

Anne-Christianne Kentgens, MD<sup>1,2,\*</sup>, Johanna M. Kurz, PhD<sup>1,2,\*</sup>, Rebeca Mozun, PhD<sup>3,4</sup>, Jakob Usemann, PhD<sup>1,5,6</sup>, Eva S. L. Pedersen, PhD<sup>3</sup>, Claudia E. Kuehni, Prof<sup>1,3</sup>, Philipp Latzin, Prof<sup>1</sup>, Alexander Moeller, Prof<sup>5</sup>, Florian Singer, PhD<sup>1,5,7</sup>, The LuftiBus In the School (LUIS) study group<sup>8</sup>

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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<sub>1</sub> 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<sub>6</sub> sulfur-hexafluoride

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**Author contributions:** ACK, JMK and FS had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, including and especially any adverse effects. ACK, JMK, RM, JU, ESLP, CEK, PL, AM, and FS contributed substantially to the study design, data analysis and interpretation, and the writing of the manuscript.

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SIII phase III slope

SnIII phase III slope normalized for expired tidal volume

SD standard deviations

SF<sub>6</sub> 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<sub>6</sub>) and the phase III slope (SIII<sub>He-SF<sub>6</sub></sub>) is derived. We assessed feasibility, repeatability, and associations of SIII<sub>He-SF<sub>6</sub></sub> 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<sub>1</sub>), and FEV<sub>1</sub>/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<sub>He-SF<sub>6</sub></sub> was independently but weakly positively associated with age and BMI. In 276 (21.2%) children, wheeze was reported. SIII<sub>He-SF<sub>6</sub></sub> was higher by 0.049 g.mol.L<sup>-1</sup> 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<sub>He-SF<sub>6</sub></sub> was not associated with FeNO, FEV<sub>1</sub>, and FEV<sub>1</sub>/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

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

10 DTG-SBW may be a simple and accessible tool to allow for early detection of lung function  
11 alterations, *i.e.* ventilation inhomogeneity, associated with negative respiratory disease outcomes.  
12 However, in unselected pediatric populations, feasibility and repeatability of the DTG-SBW, and  
13 predictors of the SIII<sub>He-SF6</sub> are unknown. Possible predictors of ventilation inhomogeneity constitute  
14 age, sex, body composition, wheeze, airflow limitation and airway inflammation<sup>7-9</sup>. Previous studies  
15 suggest that high body mass index (BMI) is associated with dysanaptic lung growth, a non-  
16 proportional growth of the airways and lung, as adipose tissue and pro-inflammatory mediators  
17 affect lung growth and development. Pediatric wheeze and airflow limitation increase the risk of  
18 chronic obstructive pulmonary disease in adults<sup>10</sup>.

19 The current study addressed two research questions: Is the DTG-SBW measurement  
20 feasible in a pediatric field study setting? What are the predictors of the DTG-SBW derived  
21 ventilation inhomogeneity estimate in a sample of school children? To accomplish this, we applied  
22 the DTG-SBW test in a large pediatric field study to assess its feasibility and reliability, and explore  
23 associations between SIII<sub>He-SF6</sub> and anthropometric variables, wheeze, and standard lung function  
24 indices.

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



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

30 We further hypothesized that the  $S_{III_{He-SF_6}}$  is associated with age and body composition<sup>7</sup>,  
31 wheeze<sup>9,16</sup>, spirometry indices, and fraction of exhaled nitric oxide (FeNO).

32

### 33 **STUDY DESIGN AND METHODS**

34 LuftiBus in the School (LUIS) is a prospective cross-sectional observational field study in  
35 unselected school-aged children (ClinicalTrials.gov: NCT03659838)<sup>17</sup>. Inclusion criteria were age  
36 six to 17 years, German language skills, and consent to participate. There were no predefined  
37 exclusion criteria. A mobile lung function-testing unit (motorbus) visited 37 schools in the canton of  
38 Zurich, the most populated canton in Switzerland, between 2013 and 2016<sup>17</sup>. Most children were  
39 born in Switzerland (88%) and predominantly of white European ancestry (75.8%). The distribution  
40 of the Swiss socioeconomic position index (Swiss-SEP) for families participating in the study was  
41 representative to the Swiss-SEP distribution from families with at least one child living in the  
42 household from the canton of Zurich<sup>17</sup>. LUIS took place throughout different seasons (e-Figure 1).  
43 A consecutively recruited convenience sample of the whole population was studied, as the  
44 hardware for DTG-SBW including tracer gas supply became available later during the study. Details  
45 about study design, sample size estimates, and data collection have been described recently<sup>17</sup>.  
46 Children performed lung function tests in the following sequence: DTG-SBW, FeNO measurement,  
47 spirometry. The ethics committee of the canton of Zurich approved the study (KEK-ZH-Nr: 2014-  
48 0491). Parents or caregivers signed the informed consent form. Children assented verbally and  
49 those aged  $\geq 15$  years also signed the informed consent form.

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

54 Tidal DTG-SBW was performed in triplet using the Exhalyzer D® (EcoMedics AG, Duernten,  
55 Switzerland) according to recommendations<sup>18</sup>. An inert double-tracer gas mixture containing 5%  
56 SF<sub>6</sub>, 26.3% He, 21% oxygen and balance nitrogen was inhaled during a single tidal breath and  
57 tidally exhaled to functional residual capacity. The setup, protocol and quality control criteria were  
58 in accordance with the *European Respiratory Society* consensus on inert gas washout testing and  
59 were previously described<sup>3,17,18</sup>. The DTG-SBW was analyzed automatically followed by quality  
60 control using a customized software platform (LungSim based on Matlab® (R2014a, The  
61 Mathworks Inc. Natick, MA, USA)<sup>17</sup>. Quality control was performed by two trained lung function  
62 technicians and included central over-read. The DTG-SBW trials were categorized according to the  
63 quality control (qc) categories A, B or failed (F). The quality control protocol used can be found in  
64 the online supplement (e-Table 1). Only children who achieved at least two acceptable DTG-SBW  
65 trials were included.

66 The primary outcome measure was the mean  $S_{III_{He-SF_6}}$  of all technically acceptable DTG-  
67 SBW curves of each subject. The  $S_{III_{He-SF_6}}$  was computed from the volumetric expirogram by fitting  
68 a linear regression slope to the molar mass signal between 65 and 95 % of the expired volume. In  
69 addition, the  $S_{III_{He-SF_6}}$  was normalized for expired volume by multiplication with the expired tidal  
70 volume ( $S_{nIII_{He-SF_6}}$ ) as a secondary outcome<sup>17</sup>. Findings are reported in the online supplement.  
71 Both lower as well as higher  $S_{III_{He-SF_6}}$  values as compared to a healthy reference population have  
72 been shown to be associated with ventilation inhomogeneity arising in central or peripheral airways,  
73 respectively<sup>2-6</sup>.

74 Fraction of exhaled nitric oxide (FeNO, parts per billion, ppb) was measured according to  
75 recommendations using a single-breath online method and a fast response chemiluminescence  
76 analyzer (CLD 88, EcoMedics AG)<sup>19</sup>. Further details on test performance and quality control were  
77 previously described<sup>17</sup>. The FeNO is a proxy of eosinophilic airway inflammation, FeNO values  $\geq$   
78 20 ppb can be considered elevated<sup>20</sup>.

79

80 Spirometry was performed using a standard spirometer (Masterlab, Jaeger, Wuerzburg,  
81 Germany) according to recommendations<sup>21</sup>. Indices were forced expiratory volume in the first  
82 second (FEV<sub>1</sub>), and the ratio of FEV<sub>1</sub> over the forced vital capacity (FEV<sub>1</sub>/FVC). Values were  
83 expressed as z-score according to *Global Lung Initiative* reference equations<sup>17,22</sup>. Lower limit of  
84 normal of FEV<sub>1</sub> and FEV<sub>1</sub>/FVC were set at  $\leq -1.645$  z-score as recommended<sup>21,22</sup>.

85

### 86 *Analysis*

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

95 Associations were assessed using scatterplots, Pearson's correlations, and univariable  
96 linear regression models. Potential predictors of SIII<sub>He-SF6</sub> included age, sex, height, weight and  
97 BMI z-score, wheeze, and FeNO, FEV<sub>1</sub> and FEV<sub>1</sub>/FVC. A multivariable linear regression model  
98 was used to explore these variables as independent predictors of SIII<sub>He-SF6</sub>. Variables were  
99 analyzed as continuous variables with their original scale, wheeze as a binary variable (*i.e.* yes or  
100 no), and FeNO as quintiles ensuring balanced observations per category. Regression model  
101 diagnostics were used to confirm underlying assumptions. P-values < 0.05 were considered  
102 statistically significant. All analyses were performed using STATA (StataCorp LP, College Station,  
103 TX, USA Version 16.0). Figures were made using GraphPad Prism version 8.0.1 (GraphPad  
104 Software, San Diego, CA, USA).

105

106

107

108 **RESULTS**

109 In total, 3870 children were enrolled into the LUIS study. The children`s median [IQR] age was 12.1  
110 [9.3-14.0] years and half of the population was female. The DTG-SBW test was applied in 1782  
111 (46.0%) children, which were slightly younger (0.7 years), had slightly lower Swiss-SEP (1.3  
112 points), reported hay fever somewhat more frequently (2.7%), and FeNO was slightly lower (2.6  
113 ppb) compared to children not invited to perform the DTG-SBW. There were no systematic  
114 differences in anthropometric and lung function estimates between these children (e-Table 2).  
115 Anthropometric characteristics and lung function estimates can be found in Table 1 and e-Table 2.

116

117 *Feasibility and Repeatability*

118 In total, 5223 DTG-SBW trials were obtained of which 4090 trials (78.3%) were of acceptable  
119 quality. Thus, 1449 (81.3%) out of 1782 children successfully achieved DTG-SBW tests (e-Tables  
120 3-5). DTG-SBW success rate was higher than the hypothesized success rate (75%). Children with  
121 successful DTG-SBW tests were 1.1 years older, had a lower Swiss-SEP, reported wheeze more  
122 often than the children with unsuccessful tests, all other anthropometric and questionnaire data  
123 were comparable (e-Table 4).

124 In children with a successful DTG-SBW test, trial quality was rated higher more often.  
125 Frequency of higher trial quality control categories was associated with the number of acceptable  
126 trials (e-Table 7, 8, and e-Figure 2) until a maximum of 4 trials. The mean (SD)  $S_{III_{He-SF_6}}$  was -0.30  
127 (0.42) g.mol.L<sup>-1</sup>. The repeatability of  $S_{III_{He-SF_6}}$  with a median [IQR] intra-test coefficient of variation  
128 of 39.8 [22.0-70.9]% was poorer than the hypothesized repeatability (25%). For more details on  
129 DTG-SBW feasibility and repeatability we refer to the online supplement (e-Figure 3, e-Table 9).

130

### 131 *Predictors of Ventilation Inhomogeneity*

132 The  $S_{III_{He-SF_6}}$  was associated with all preselected anthropometric variables except for sex. In  
133 univariable regression models,  $S_{III_{He-SF_6}}$  was positively associated with age, height, weight, and  
134 BMI z-score (Table 2, Figure 2). In a multivariable regression model, only age and BMI remained  
135 independent predictors of  $S_{III_{He-SF_6}}$ , increasing  $S_{III_{He-SF_6}}$  by  $0.013 \text{ g.mol.L}^{-1}$  per one year increase in  
136 age and by  $0.060 \text{ g.mol.L}^{-1}$  per one z-score increase in BMI, respectively.

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

141 FeNO was slightly higher (4.3 ppb), and spirometry lower ( $FEV_1$ , 0.21 z-score) than in  
142 children without wheeze (e-Table 10). The  $S_{III_{He-SF_6}}$  was associated with wheeze in univariable  
143 regression models, and remained weakly positively associated with wheeze after adjustment for  
144 age and BMI z-score (Table 2).  $S_{III_{He-SF_6}}$  was higher by  $0.049 \text{ g.mol.L}^{-1}$  in children with wheeze as  
145 compared to those without), but was not associated with FeNO or with the spirometry indices  $FEV_1$ ,  
146 and  $FEV_1/FVC$  (Table 2, e-Table 11). A *post hoc* analysis in a sub-group of children with a BMI z-  
147 score  $>1.0$  showed similar results as compared to the primary analysis in the whole cohort (e-Table  
148 12).

149

## 150 **DISCUSSION**

151 In this large pediatric field study setting, we found that the DTG-SBW measurement was feasible  
152 in a mobile bus lung function laboratory. Repeatability was poorer than hypothesized. We identified  
153 predictors of ventilation inhomogeneity in unselected schoolchildren.  $S_{III_{He-SF_6}}$  was weakly  
154 positively associated with age, BMI and wheeze but not with FeNO or spirometry indices. On the  
155 population level in sufficiently large samples such as ours, the  $S_{III_{He-SF_6}}$  captures a subtle signal of  
156 alterations in ventilation inhomogeneity suggesting small airways dysfunction in children with  
157 wheeze. However, on the individual level, the  $S_{III_{He-SF_6}}$  does not seem sensitive enough to screen  
158 for alterations in ventilation inhomogeneity in unselected children.

159

160 *Interpretation*

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

173 The coefficient of variation quantifying intra-test variability of  $S_{III_{He-SF_6}}$  was higher than  
174 previously reported (19%) for DTG-SBW<sup>2</sup> but comparable to the SIII indices  $S_{cond}$  and  $S_{acin}$  from  
175 the established multiple-breath washout test supporting the reliability of the current analysis<sup>6,24</sup>.  
176 The estimated mean value of  $S_{III_{He-SF_6}}$  was close to zero in our study, therefore small changes may  
177 have increased the coefficient of variation exponentially. The variability seen can be due to factors  
178 related to the field-study setting, but estimation of the proportion of variability that can be attributed  
179 to the setting, is challenging. It is well established, however, that the intra-test variability for inert  
180 gas analysis is high, commonly thought to be due to effects of breathing. Interestingly, variability of  
181  $S_{III_{He-SF_6}}$  was associated with age and the variability in tidal volume in our study, but not with other  
182 potential explanatory variables, such as the  $S_{III_{He-SF_6}}$  value itself. These data suggest that SIII  
183 indices are prone to considerable inherent physiological variability, and tidal breathing.  
184 Normalization for tidal volume alone may not substantially decrease variability or increase  
185 sensitivity of the test<sup>22,25,26</sup>. Current protocols for SIII measurement seem to require refinement prior

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

190 Additionally, previous data demonstrated that  $S_{III_{He-SF_6}}$  correlates with standard estimates  
191 of ventilation inhomogeneity<sup>2-6,24</sup>. However, it is unclear whether  $S_{III_{He-SF_6}}$  is also a proxy of  
192 structural airway disease. While it is established that in Cystic Fibrosis, the lung clearance index  
193 correlates with structural airway changes detected in chest computed tomography, there is one  
194 negative study for the  $S_{III_{He-SF_6}}$ <sup>27</sup>. Multiple-breath washout or lung imaging were not obtained in this  
195 field study. Yet these estimates would have allowed more in-depth assessment of the diagnostic  
196 performance of  $S_{III_{He-SF_6}}$ . Our study provides further evidence, that body composition is a predictor  
197 of lung function development. Our data are in line with previous findings suggesting age- or height-  
198 dependent effects on ventilation inhomogeneity estimates such as lung clearance index from  
199 multiple-breath washout<sup>7,28</sup>. Our data further suggest that unfavourable body composition  
200 estimated by BMI may modify ventilation inhomogeneity. Reasons remain speculative but may  
201 partly relate to airway dysanapsis observed in children with high BMI<sup>28</sup>. Indeed, we have recently  
202 shown that the spirometry indices obtained in this cohort did not fit well the reference values from  
203 the *Global Lung Function Initiative*<sup>26</sup>. Underestimation of  $FEV_1$  and FVC in the current cohort was  
204 partly explained by BMI, though  $FEV_1/FVC$  was not affected.

205 Wheezy symptoms are common and account for considerable burden in pediatric health  
206 care. We found altered ventilation inhomogeneity possibly arising in obstructed small airways  
207 related to previous wheezy symptoms<sup>2,4-6</sup>. Interestingly, our study suggests that these alterations  
208 in ventilation inhomogeneity were independent of airway inflammation or airflow limitation.  
209 However, overlap in  $S_{III_{He-SF_6}}$  values of children with vs without wheeze was considerable.  
210 Comparable to other studies, peripheral airway function estimated by current inert gas tests  
211 appears largely normal in children with wheeze<sup>29</sup>. Therefore, the difference in  $S_{III_{He-SF_6}}$  in children  
212 with wheeze was relatively small and adjustment for age and BMI further increased the confidence

213 intervals. Comparable to  $S_{III_{He-SF_6}}$ , FeNO, FEV<sub>1</sub> and FEV<sub>1</sub>/FVC values were overlapping between  
214 children with vs without wheeze suggesting overall relatively low pre-test probability (*i.e.* low  
215 prevalence) of lung function abnormalities in the current cohort.

216

### 217 *Strengths and Limitations*

218 The large sample size is a strength of this prospective study, as it allows conclusive analyses of  
219 potential predictors of lung function. Our study allowed for thorough assessment of potential  
220 predictors of the  $S_{III_{He-SF_6}}$  estimate, including anthropometric and lung function measures. The large  
221 sample of unselected schoolchildren supports the generalizability of our findings. Participation of  
222 schools was decided by the heads of the schools which may have introduced selection to some  
223 extent. Yet, the Swiss-SEP for families participating in the study was representative for the canton  
224 of Zurich<sup>11</sup>. As the DTG-SBW test was introduced later in this study, only a subgroup of the LUIS  
225 study was invited to perform DTG-SBW. During this study period, the frequency of measurements  
226 varied over time. The  $S_{III_{He-SF_6}}$  was not influenced by timing of measurements, *i.e.* seasonal effects.

227 The current protocol determined the sequence of testing to avoid influences from forced  
228 breathing manoeuvres during spirometry on  $S_{III_{He-SF_6}}$  and FeNO. Tidal inhalation of inert gas during  
229 the DTG-SBW unlikely influenced subsequent FeNO or spirometry measurements.

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

235 The proportion of variation in  $S_{III_{He-SF_6}}$  in this unselected population, that can be explained  
236 by wheeze, was low. We acknowledge that questionnaire-based classification of wheeze may have  
237 been subject to recall and misclassification bias. Parent reported wheeze may have been less  
238 precise compared to physician reported wheeze. The sound of wheezing that parents notice  
239 unaided by a stethoscope (*i.e.* “audible” wheeze) originates from trachea and larger bronchi, rather



240 than from the peripheral small airways. We assume that misclassification rather led to  
241 underestimation of the strength of association between wheeze and  $SIII_{He-SF_6}$ . Premature birth may  
242 affect lung development and alter ventilation inhomogeneity in some children. We were unable to  
243 explore possible effects of prematurity on  $SIII_{He-SF_6}$ .

244

#### 245 *Outlook*

246 Our results suggest that DTG-SBW is feasible in children between six and 17 years of age. Data  
247 from younger children are scant and warrant further study<sup>2,4,30</sup>. Despite of good feasibility, the high  
248 variability and presumably low sensitivity to capture slightly increased ventilation inhomogeneity  
249 constrain its use in unselected individuals. Currently, the DTG-SBW is applicable in research  
250 settings and sufficiently large populations or in selected individuals with high pre-test probability of  
251 lung function abnormalities. In the latter, we have shown that the  $SIII_{He-SF_6}$  is responsive to  
252 bronchodilator inhalation in asthma or chest physiotherapy in cystic fibrosis<sup>2-6</sup>. Distinct  
253 interpretation of dynamics in  $SIII_{He-SF_6}$  warrants further research. Future longitudinal studies are  
254 warranted to establish the minimal clinically important difference derived from variability estimates  
255 and patient reported outcomes.

256 To conclude, the DTG-SBW measurement is feasible in pediatric field studies. However, relatively  
257 high variability of  $SIII_{He-SF_6}$  appears to limit the interpretation. This makes DTG-SBW currently  
258 unsuitable in small populations with low pre-test probability of impaired lung function. In the current  
259 relatively large population of unselected schoolchildren, age, body composition and wheeze were  
260 identified as predictors of ventilation inhomogeneity estimated by the  $SIII_{He-SF_6}$ . Schoolchildren with  
261 wheeze may have alterations in ventilation inhomogeneity which can be attributed to peripheral  
262 airway dysfunction.

263

#### 264 **Take-Home Points**

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

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

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

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**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
<b>General characteristics</b>			
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]
<b>Lung function</b>			
FeNO (ppb), median (IQR)	12.3 (7.2–21.5)	11.0 (6.3-19.6)	11.1 (6.1-19.7)
FEV <sub>1</sub> (z-score)	-0.5 (1.0)	-0.52 (0.97)	-0.54 (0.97)
FEV <sub>1</sub> /FVC (z-score)	-0.2 (1.1)	-0.25 (1.04)	-0.24 (1.06)
SIII <sub>He-SF6</sub> (g.mol.L <sup>-1</sup> )		-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<sub>1</sub>: forced expired volume in the first second, FVC: forced vital capacity, SIII<sub>He-SF6</sub>: DTG-SBW slope of phase III.

**Table 2** Non-adjusted and adjusted association between  $S_{III_{He-SF_6}}$  and potential predictors

<b>Predictors</b>	<b>Regression coefficients</b>	<b>95% CI</b>	<b>P-value*</b>
<b>Anthropometrics</b>			
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*
<b>Symptoms</b>			
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
<b>Lung function</b>			
FeNO (quintiles )	0.004	-0.010 to 0.018	0.557
FEV <sub>1</sub> (z-score)	0.012	-0.008 to 0.032	0.255
FEV <sub>1</sub> /FVC (z-score)	0.005	-0.013 to 0.023	0.606

Associations between  $S_{III_{He-SF_6}}$  and potential predictors were assessed using uni- and multivariable linear regression models. Predictors were age, sex, height, weight and BMI; wheeze, FeNO, FEV<sub>1</sub> and FEV<sub>1</sub>/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  $S_{III_{He-SF_6}}$ , and the independent predictors age and BMI were used to adjust the association of  $S_{III_{He-SF_6}}$  with wheeze. All associations described the change in  $S_{III_{He-SF_6}}$  in g.mol.L<sup>-1</sup> 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,  $S_{III_{He-SF_6}}$ : slope of phase III,  $Sn_{III_{He-SF_6}}$ : normalized  $S_{III_{He-SF_6}}$ , BMI: body mass index (z-score). FeNO: fraction of exhaled nitric oxide, FEV<sub>1</sub>: forced expired volume in the first second, FVC: forced vital capacity.

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

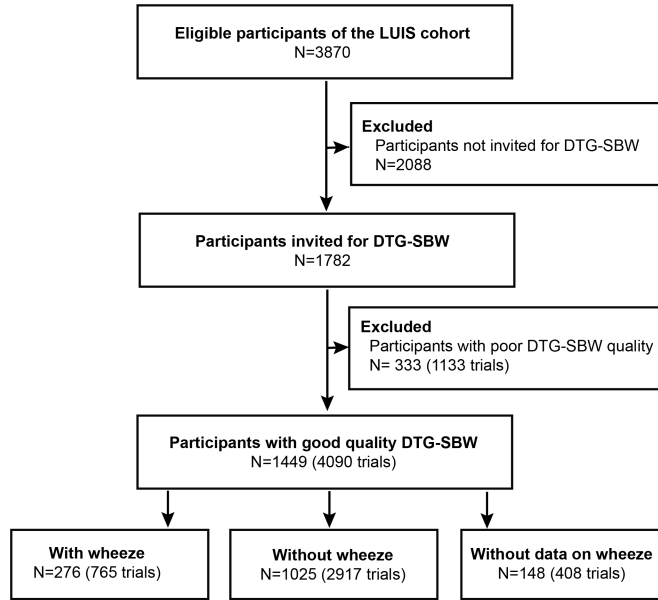
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366 **Figure 2** Scatterplot of the double-tracer gas (helium sulfur-hexafluoride) single-breath washout  
367 derived phase III slope ( $S_{III_{He-SF_6}}$ ) vs. body mass index (BMI, left panel a) and forced expiratory  
368 volume in the first second (FEV1, right panel b). BMI and FEV1 are expressed as z-score. The  
369 closed circles display  $S_{III_{He-SF_6}}$  values of children without wheeze and open circles values of  
370 children with wheeze. We have excluded one outlier (BMI = -1.7 z-score and  $S_{III_{He-SF_6}} = 2.9$  g.mol.L-  
371 1) in figure panel a) to ease visualization.

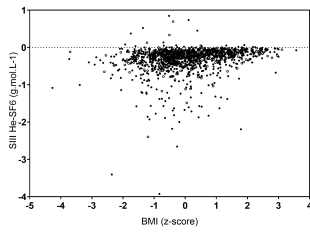
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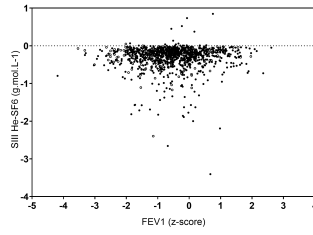
Journal Pre-proof



a)



b)



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