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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

FEV₁ 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

SF₆ 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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Abbreviations

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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

SF₆ 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 (SF₆) 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 (FEV₁), and FEV₁/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, FEV₁, and FEV₁/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 periphery¹. 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 (SF₆)^{2,3}. The derived slope of phase III (SIII_{He-SF6}) measures ventilation
7 inhomogeneity of He and SF₆ which differ in diffusive gas mixing properties in small airway
8 compartments^{2,3}. The SIII_{He-SF6} measurement is reliable in research settings and captures altered
9 ventilation inhomogeneity in children with asthma or cystic fibrosis²⁻⁶.

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_{He-SF6} are unknown. Possible predictors of ventilation inhomogeneity constitute
14 age, sex, body composition, wheeze, airflow limitation and airway inflammation⁷⁻⁹. 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 adults¹⁰.

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_{He-SF6} 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^{11,12}. For multiple breath washout, the success
27 rates ranged between 50-100% in children¹³⁻¹⁵. 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 composition⁷,
31 wheeze^{9,16}, 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)¹⁷. 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 2016¹⁷. 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 Zurich¹⁷. 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 recently¹⁷.
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 ≥ 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 medication¹⁷.
52 Wheeze was specified as parental report of continuous whistling sound during expiration during
53 one or more episodes in the past 12 months¹⁷.

54 Tidal DTG-SBW was performed in triplet using the Exhalyzer D® (EcoMedics AG, Duernten,
55 Switzerland) according to recommendations¹⁸. An inert double-tracer gas mixture containing 5%
56 SF₆, 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^{3,17,18}. 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)¹⁷. 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 SIII_{He-SF₆} of all technically acceptable DTG-
67 SBW curves of each subject. The SIII_{He-SF₆} 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 SIII_{He-SF₆} was normalized for expired volume by multiplication with the expired tidal
70 volume (SnIII_{He-SF₆}) as a secondary outcome¹⁷. Findings are reported in the online supplement.
71 Both lower as well as higher SIII_{He-SF₆} 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 respectively²⁻⁶.

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)¹⁹. Further details on test performance and quality control were
77 previously described¹⁷. The FeNO is a proxy of eosinophilic airway inflammation, FeNO values ≥
78 20 ppb can be considered elevated²⁰.

79

80 Spirometry was performed using a standard spirometer (Masterlab, Jaeger, Wuerzburg,
81 Germany) according to recommendations²¹. Indices were forced expiratory volume in the first
82 second (FEV₁), and the ratio of FEV₁ over the forced vital capacity (FEV₁/FVC). Values were
83 expressed as z-score according to *Global Lung Initiative* reference equations^{17,22}. Lower limit of
84 normal of FEV₁ and FEV₁/FVC were set at ≤ -1.645 z-score as recommended^{21,22}.

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 imputed¹⁷. 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_{He-SF6} included age, sex, height, weight and
97 BMI z-score, wheeze, and FeNO, FEV₁ and FEV₁/FVC. A multivariable linear regression model
98 was used to explore these variables as independent predictors of SIII_{He-SF6}. 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⁻¹. 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 settings². 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 findings²³. 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 children².
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 errors².

173 The coefficient of variation quantifying intra-test variability of $S_{III_{He-SF_6}}$ was higher than
174 previously reported (19%) for DTG-SBW² 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^{6,24}.
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^{22,25,26}. 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^{2-6,24}. 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 $S_{III_{He-SF_6}}$ ²⁷. 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^{7,28}. 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 BMI²⁸. 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*²⁶. 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^{2,4-6}. 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 wheeze²⁹. 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, FEV₁ and FEV₁/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 Zurich¹¹. 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^{2,4,30}. 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 fibrosis²⁻⁶. 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-	With acceptable DTG-
		SBW	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, FEV₁: forced expired volume in the first second, FVC: forced vital capacity, SIII_{He-SF6}: DTG-SBW slope of phase III.

Table 2 Non-adjusted and adjusted association between $S_{III_{He-SF_6}}$ 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 $S_{III_{He-SF_6}}$ and potential predictors were assessed using uni- and multivariable linear regression models. Predictors were age, sex, height, weight and BMI; wheeze, FeNO, FEV₁ and FEV₁/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⁻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, $S_{III_{He-SF_6}}$: 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, FEV₁: forced expired volume in the first second, FVC: forced vital capacity.

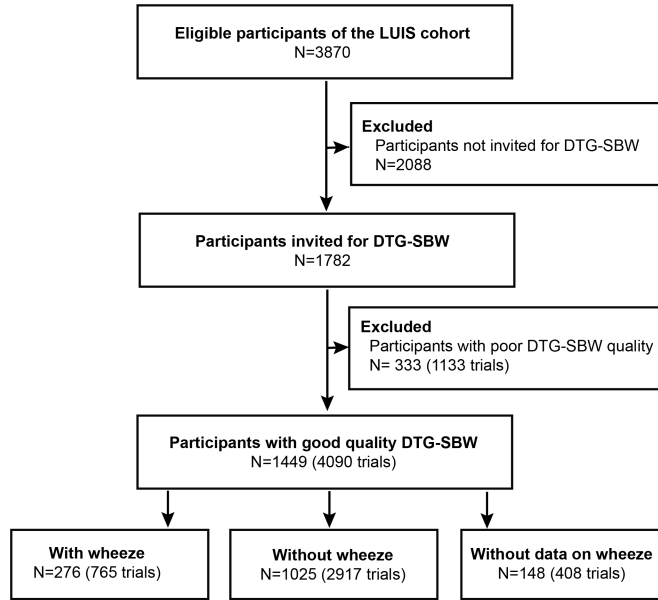
361 **Figure 1** Flow chart of study participants and success rate of DTG-SBW. Out of the 3870 children
362 of the LUIS study, 1782 children performed DTG-SBW (46%). Of these children, 1449 children had
363 acceptable DTG-SBW data (81%). LUIS study: LuftiBus in the school study, DTG-SBW: Double-
364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

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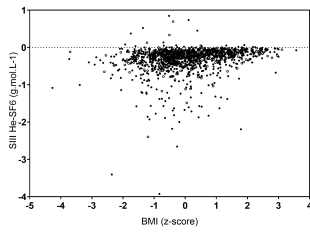
366 **Figure 2** Scatterplot of the double-tracer gas (helium sulfur-hexafluoride) single-breath washout
367 derived phase III slope ($S_{III_{He-SF_6}}$) vs. body mass index (BMI, left panel a) and forced expiratory
368 volume in the first second (FEV1, right panel b). BMI and FEV1 are expressed as 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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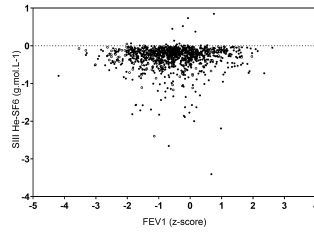
Journal Pre-proof



a)



b)



Journal Pre-proof