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Evaluation of interrupter resistance in methacholine challenge testing in children

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8 3 **Authors:** Marije Koopman, MD¹; Hein J.L. Brackel, MD, PhD²; Anja A.P.H. Vaessen-
9
10 4 Verberne, MD, PhD³; Wim C. Hop, PhD⁴; Cornelis K. van der Ent, MD, PhD¹; on behalf of
11
12 5 the COMBO-Rint research group.
13
14 6

15
16
17 7 **Institutions:**

- 18
19
20 8 1. Department of Paediatric Pulmonology, Wilhelmina Children's Hospital, University
21
22 9 Medical Centre Utrecht, Utrecht, the Netherlands.
23
24 10 2. Department of Paediatrics, Catharina Hospital, Eindhoven, the Netherlands.
25
26 11 3. Department of Paediatrics, Amphia Hospital, Breda, the Netherlands
27
28 12 4. Department of Biostatistics, Erasmus MC – University Medical
29
30 13 Centre Rotterdam, Rotterdam, the Netherlands
31
32
33
34 14

35
36 15 **COMBO-Rint research group consists of:** H.G.M. Arets, N.J. van den Berg, P.L.P. Brand,
37
38 16 E.J. Duiverman, H.J. Hendriks, J.W.C.M. Heynens, J.C. van Nierop, M. Nuysink.
39
40
41 17

42
43 18 **Corresponding author**

44
45 19 Ms. Marije Koopman

46
47 20 Wilhelmina Children's Hospital/University Medical Centre Utrecht

48
49 21 Department of Paediatric Pulmonology, Huispostnummer KH.01.419.0

50
51 22 P O Box 85090, 3508 AB Utrecht, the Netherlands

52
53 23 Email: M.Koopman-5@umcutrecht.nl

54
55 24 Telephone number: 0031-88-7553201 / Fax number: 0031-88-7554747
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20 33 **Running head:** R_{int} in bronchial challenge testing
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3 34 **SUMMARY**
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9 37 Bronchial hyperresponsiveness is a key feature of asthma and is assessed using bronchial
10 38 provocation tests. The primary outcome in such tests (a 20% decrease in forced expiratory
11 39 volume in 1 second (FEV₁)) is difficult to measure in young patients. This study evaluated the
12 40 sensitivity and specificity of the interrupter resistance (R_{int}) technique, which does not require
13 41 active patient participation, by comparing it to the primary outcome measure.
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18 41 Methacholine challenge tests were performed in children with a history of moderate
19 42 asthma and bronchial hyperresponsiveness. Mean and individual changes in R_{int} and FEV₁
20 43 were studied. A receiver operating characteristic (ROC) curve was used to describe sensitivity
21 44 and specificity of R_{int}.
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28 45 Seventy-three children (median age: 9.2 yrs; range: 6.3- 13.4 yrs) participated. There
29 46 was a significant (p< 0.01) increase in mean R_{int} with increasing methacholine doses.
30 47 However, individual changes of R_{int} showed large fluctuations. There was great overlap in
31 48 change of R_{int} between children who did and did not reach the FEV₁ endpoint. A ROC-curve
32 49 showed an area under the curve of 0.65.
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40 50 Because of low sensitivity and specificity, the use of R_{int} to diagnose bronchial
41 51 hyperresponsiveness in individual patients seems limited.
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55 55 **Key words:** Bronchial hyperresponsiveness, bronchial provocation test, forced expiratory
56 56 volume in 1 second, interrupter resistance, methacholine challenge test, sensitivity,
57 57 specificity.
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59 INTRODUCTION

60 According to GINA guidelines bronchial hyperresponsiveness (BHR) is a key feature of
61 asthma (1). To objectify BHR, forced flow volume manoeuvres are required to measure the
62 forced expiratory volume in one second (FEV_1), as primary outcome measure in bronchial
63 challenge testing (2). Because of the difficulty of obtaining forced lung function
64 measurements in early childhood the diagnosis of asthma in this age group is challenging and
65 complicated. Several studies have advocated measurement of the interrupter resistance (R_{int})
66 as an alternative to quantify airway obstruction in preschool children and in children with
67 developmental delay (3;4). The interrupter technique is non-invasive and can be obtained
68 during tidal breathing (5). R_{int} can be measured in children from two years of age as it
69 requires minimal cooperation (6). The R_{int} technique has been compared to body
70 plethysmography (7) and transcutaneous partial pressure of oxygen measurements (7-10)
71 during methacholine challenge tests, but most studies made no comparison with the outcome
72 measure FEV_1 . The one study that compared R_{int} with FEV_1 during methacholine challenge
73 testing reported a mean increase in R_{int} with increasing doses of methacholine (11). However,
74 this study did not present the changes in R_{int} for individual patients nor how these related to
75 individual changes in FEV_1 . The ability of R_{int} to detect methacholine induced
76 bronchoconstriction in individual patients, as measured by a 20% decrease in FEV_1 , has never
77 been studied.

78 The latest ATS/ERS statement on pulmonary function testing in preschool children
79 recommended the need to determine the role of R_{int} in bronchial challenge tests (5). The
80 current study evaluated the sensitivity and specificity of R_{int} to detect airflow obstruction
81 during methacholine challenge tests in asthmatic children by comparing measurements with
82 FEV_1 as the gold standard.

83 MATERIALS AND METHODS

84 This study was a substudy of the multicentre COMBO-study in children between 6 and 16
85 years with moderate asthma (see online supplement) (12). Nine out of 19 hospitals performed
86 additional R_{int} measurements during methacholine challenge tests. The children from these
87 centres were included in this substudy. It was approved by the local medical ethical
88 committees and written informed consent was obtained from the parents (and the child if ≥ 12
89 years) before start of the study. For additional information see online supplement.

91 Study protocol

92 Methacholine provocation tests were performed twice (at start of study and after 26 weeks)
93 measuring R_{int} and FEV_1 after each methacholine dose. All children abstained from long-
94 acting β_2 agonists and inhaled steroids for 12 hours before the first methacholine provocation
95 test (and 36 hours before the second methacholine provocation test), and from short acting β_2
96 agonists for 6 hours before provocation testing. To prevent possible, unwanted physiologic
97 effects of deep inhalation on R_{int} , R_{int} was measured prior to FEV_1 . Pulmonary function tests
98 were performed to conform with latest ATS/ERS guidelines (2;5;13) and briefly described
99 below. The complete methodology is described in the online supplement.

101 *Methacholine challenge testing*

102 Methacholine challenge tests were performed using the dosimeter method (2;14) with a
103 nebulizer (model 646, DeVilbiss Healthcare, Somerset, USA) attached to a dosimeter
104 (Rosenthal French dosimeter, PDS Instrumentation, Louisville CO, USA). Methacholine
105 chloride was administered in doubling doses and provocation was continued after at least five
106 minutes and until the dose at which at least 20% fall in FEV_1 (PD_{20}) or the maximum dose
107 was reached.

108

109 *Interrupter resistance*

110 Airway resistance measurements were carried out using the MicroRint (Micro Medical
111 Limited, Kent, UK). A bacterial filter was used (Spirosafe bacterial filter, Micro Medical,
112 Kent, UK). Reference values of Merkus et al. (15) were used.

113

114 *Spirometry*

115 Maximal expiratory flow-volume measurements were performed with standardized
116 equipment, using reference values of Stanojevic (16).

117

118 *Analysis*

119 Changes in FEV₁ and R_{int} were analyzed as percentages change from baseline. Change in R_{int}
120 was also assessed as change in percentage predicted and change in z-score, computed from
121 the latest R_{int} reference values (15). Wilcoxon Signed Ranks test was used to investigate
122 whether there was a significant increase in mean change from baseline of R_{int} at each dose
123 step. To quantify the variability of FEV₁ and R_{int} within individuals between the various
124 dosesteps, coefficients of variation (CV) were calculated (see online supplement for
125 calculation). The resulting individual paired CV's of FEV₁ and R_{int} were compared using
126 Wilcoxon's test. Comparison of mean CV's between centers was done using the Kruskal-
127 Wallis test. The association between changes in FEV₁ and changes in R_{int} at PD₂₀ was
128 analysed using Spearman's correlation (r_s). At each separate dose step Mann Whitney tests
129 were computed to compare the difference in changes from baseline between subjects who did
130 and who did not reach the PD₂₀ endpoint at the dose step. Cox regression, with change from
131 baseline of R_{int} as time-dependent variable, was used to analyse whether these changes were
132 predictive for having reached the PD₂₀ endpoint at the subsequent dose steps. The sensitivity

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3 133 and specificity of R_{int} in relation to having reached FEV_1 -based PD_{20} was described by
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5 134 receiver operating characteristic (ROC) curves. The area under the curve (AUC) shows
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8 135 whether the change in R_{int} can correctly classify those who do and do not reach the PD_{20}
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10 136 endpoint. We used the coefficient of repeatability calculated by Beydon et al. (a 32.1%
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12 137 increase in R_{int}) as a cut-off value to calculate the sensitivity and specificity of R_{int} to predict a
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15 138 20% fall in FEV_1 .
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142 RESULTS

143 At the first COMBO-study visit 73 children with moderate asthma (60 % boys) with a median
144 age of 9.2 years (range: 6.3 – 13.4 years) performed both R_{int} and FEV_1 measurements during
145 methacholine challenge. Baseline characteristics are shown in Table 1. Fifty-two children
146 reached the PD_{20} endpoint and were defined as responders (Table 2).

147 There was a significant increase from baseline of R_{int} starting at the sixth methacholine
148 dose step and all dose steps thereafter (all $p < 0.01$). From the first dose step mean FEV_1 was
149 significantly different from baseline value (all $p < 0.01$), except for dose step 2. Visual
150 inspection of individual R_{int} profiles during the challenge test showed large fluctuations with
151 rises and falls at subsequent dose steps. In contrast, much smoother profiles with gradual falls
152 were found for FEV_1 (see E-figure 1 in the online supplement). To express the variability in
153 individual curves, the CV of measured values was calculated per patient. For R_{int} the mean
154 CV of all patients was 10.6% (10th - 90th percentile: 5.5% – 16.3%). The mean CV for FEV_1
155 was significantly smaller: 2.9% (1.7% - 4.3%, $p < 0.001$). Further analysis of CV R_{int} showed
156 that there was no correlation with age ($r_s = -0.13$, $p = 0.26$). Also centres did not significantly
157 differ from each other (Kruskal-Wallis test: $p = 0.17$).

158 Analysing the 52 children who reached the PD_{20} endpoint, there was no significant
159 correlation between changes from baseline of FEV_1 and changes from baseline of R_{int} at the
160 last dose step at which the PD_{20} endpoint was reached ($r_s = 0.11$, $p = 0.44$).

161 Figure 1 demonstrates the mean changes from baseline of FEV_1 and R_{int} at each dose
162 step, while at each dose step patients are grouped according to whether or not the PD_{20}
163 endpoint was reached at the dose step. At none of the dose steps there was a significant
164 difference in the mean change of R_{int} from baseline between those who reached the PD_{20}
165 endpoint at that dose step versus those who did not, except at dose step 6 ($p = 0.014$) (figure
166 1B). A combined analysis of all dose steps comparing responders and non-responders, using

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3 167 Cox regression analysis, showed that a greater change of R_{int} was associated with a higher
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5 168 probability of reaching the PD₂₀ endpoint ($p= 0.038$). However, when inspecting the
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8 169 individual changes from baseline, there is large overlap of children who did and who did not
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10 170 reach the PD₂₀ endpoint at the various dose steps (Figure 2). A ROC curve of changes from
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12 171 baseline of R_{int} as a measure to detect a 20% decrease in FEV₁ is shown in Figure 3, resulting
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15 172 in a small AUC of 0.65.

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17 173 When using a 32.1% increase in R_{int} as cut-off value to calculate the sensitivity and
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19 174 specificity of R_{int} to predict a 20% fall in FEV₁, we found a sensitivity of 50% and a
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22 175 specificity of 43%. This means that R_{int} was able to detect half of the responders as
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24 176 determined by PD₂₀ and that in 12 out of 21 children R_{int} increased with more than 32%
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27 177 without a concomitant fall of FEV₁ with 20% .

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29 178 Repeating all analyses with change in R_{int} expressed as change in percentage predicted
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31 179 and change in z-score led to similar results (data not shown). AUC's of ROC curves for
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34 180 change in percentage predicted and for change in z-score were both 0.66.

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36 181 In 64 children the methacholine challenge test was repeated after 26 weeks. The
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38 182 analyses to compare R_{int} with FEV₁ (as described above) showed similar results (data not
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41 183 shown). The AUC of the ROC curve was 0.61.

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3 186 **DISCUSSION**
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5 187 This study showed that, although there was a significant increase of mean R_{int} values with
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7 188 higher doses of methacholine, individual changes in R_{int} are highly variable during
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10 189 methacholine challenge. The sensitivity and specificity of R_{int} to detect methacholine induced
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12 190 bronchoconstriction is low, which might limit the use of R_{int} in individual patients.
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15 191 Several studies evaluated lung function measures requiring less patient effort than
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17 192 FEV_1 , which could possibly be used as alternative measures of changes in
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20 193 bronchoconstriction. Klug et al showed that R_{int} was significantly less sensitive than specific
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22 194 airway resistance in detecting changes in airway function (7). Phagoo et al found that R_{int} was
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24 195 also less sensitive and showed more variability than transcutaneous partial pressure of oxygen
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26 196 measurements (9;10). In a study of Beydon et al. the majority of children with a 20% fall in
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28 197 transcutaneous partial pressure of oxygen ($PD_{20}P_{tc}O_2$) showed a significant change of R_{int}
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30 198 during methacholine challenge. However, all children who did not reach the $PD_{20}P_{tc}O_2$ had at
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32 199 least once a significant change from baseline (8), resulting in many false positive as well as
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35 200 false negative outcomes.
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38 201 Despite these data R_{int} is still advocated as an alternative measure for airway
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40 202 obstruction in those who are not able to perform forced breathing manoeuvres. Several studies
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42 203 showed significant correlation between gold standard FEV_1 and R_{int} in groups of patients
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44 204 (4;6), and reference values for R_{int} measurement in healthy controls have been published
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46 205 (15;17-20). Although there is convincing evidence on the value of R_{int} measurement as a
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48 206 measure of airway obstruction in groups of patients, the sensitivity of R_{int} to detect changes in
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50 207 airway obstruction in individual patients has been little researched. Only one previous study
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52 208 compared R_{int} with FEV_1 during methacholine challenge testing (11) and found that on
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54 209 average R_{int} increased during methacholine challenge comparable to changes in FEV_1
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56 210 measurements, but individual changes in R_{int} were not shown. Kannisto et al. compared R_{int}
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3 211 with FEV₁ during exercise challenge to diagnose BHR (21). However, in this study only 11
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5 212 (22%) children reached the R_{int} endpoint (15% increase from baseline) and only 6 of them
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8 213 also reached the FEV₁ endpoint (10% decrease from baseline). The children who showed a
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10 214 15% rise in R_{int} but who did not have a 10% decrease in FEV₁ could be misclassified by R_{int}
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12 215 as having BHR. **Davies et al. investigated the correlation between R_{int} and FEV₁ in**
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15 216 **bronchodilator response, and despite a correlation between FEV₁ and R_{int} in a group of**
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17 217 **patients, they found no significant correlation in individual children (22).**

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20 218 To evaluate the sensitivity and specificity of the R_{int} technique to detect changes in
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22 219 airway obstruction in individual children we performed simultaneous measurements of R_{int}
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24 220 and gold standard FEV₁ in a large group of children with asthma. We show that in individual
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26 221 cases the variability of R_{int} measurements is high (mean intra-patient CV 10.6%), precluding
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28 222 proper prediction of changes in FEV₁. In the present study the ROC curve, describing the
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30 223 sensitivity and specificity of R_{int} in detecting BHR as determined by the primary outcome
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32 224 FEV₁, had a small AUC of 0.65. This predictive capacity is lower compared to impulse
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34 225 oscillometry (AUC 0.68 to 0.75 (23)).

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38 226 Large individual fluctuations in R_{int} might be due to large inpatient short-term
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40 227 variation in airway resistance during tidal breathing (11;17;20;24). This variability, resulting
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42 228 in low sensitivity and specificity, could limit the ability to detect thresholds in individual
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44 229 airway obstruction as required in clinical practice. Furthermore the sensitivity might be lower
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46 230 compared to FEV₁ because during (severe) airflow obstruction equilibration of alveolar
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48 231 pressure might take longer than is realised during the shutter closure time during R_{int}
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50 232 measurement (25). This may explain the lower R_{int} values in case of (severe) peripheral
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52 233 airway obstruction and hence lead to misclassifications during methacholine challenge
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55 234 testing. A third explanation of high variability of R_{int} might be the variability of upper airway
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3 235 resistance. Proper positioning of the head and neck and adequate support of the cheeks is
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5 236 highly warranted and might lead to intra-individual variability.
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8 237 The findings of our study imply that on group level BHR can be documented, since we
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10 238 showed on average a significant rise in R_{int} with increasing methacholine doses. This may be
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12 239 useful in epidemiologic research. However, R_{int} seems to have insufficient capacity to
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14 240 diagnose BHR in individual patients and therefore it is of limited value in BHR measurements
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16 241 in clinical practice.
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19 242 A major strength of our study is the fact that the methacholine challenge test was
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21 243 repeated after 26 weeks and resulted in a similarly low sensitivity and specificity of R_{int} ,
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23 244 which validated the results. Beside this, the present study may have some limitations. Firstly,
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25 245 the study was performed in children with a mean age of 9.3 years, because preschool children
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27 246 are seldom able to perform FEV_1 measurements. It could be questioned if the found results
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29 247 are applicable to younger children. Since R_{int} is a measure of total airway resistance, an
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31 248 induced bronchoconstriction will lead to a relative larger increase in R_{int} in young children
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33 249 compared to older children, because in young children lower airways resistance contributes
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35 250 more to total airway resistance (26). Nevertheless, the fluctuations in R_{int} are expected to be
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37 251 similar as the short term repeatability of R_{int} that has been found to be independent of age
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39 252 (20;27). Additionally we used a 20% fall as 'gold standard' in detecting methacholine induced
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41 253 bronchoconstriction. When we used a 32% increase in R_{int} (the coefficient of repeatability of
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43 254 R_{int} measurements as described by Beydon et al.) as a cut-off value to determine BHR, there
44
45 255 were 12 children with a significant change in R_{int} but without a 20% fall in FEV_1 . The
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47 256 pathophysiology of a fall in FEV_1 during a forced manoeuvre might be different from an
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49 257 increase of R_{int} during tidal breathing. Therefore, there is a possibility that FEV_1 might not be
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51 258 the gold standard for bronchial challenges especially in young children.
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3 259 ~~Therefore, there could have been children with BHR, who did not have a 20% fall in~~
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5 260 ~~FEV₁ but who did have a significant increase in R_{int}. This might have resulted in too low~~
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8 261 ~~sensitivity because of too many false positives. However, FEV₁ is the primary outcome in~~
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10 262 ~~methacholine challenge test, and no better 'gold standard' is available.~~
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12 263 The current study showed on average an increase of R_{int} with subsequent methacholine
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15 264 doses during methacholine challenge tests and therefore it might be useful as a measure of
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17 265 airflow obstruction in groups of patients, e.g. in large epidemiological studies. However, we
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20 266 conclude that R_{int} seems to have insufficient capacity to diagnose BHR in individual patients.
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3 373 **FIGURE LEGENDS**
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8 375 **Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step**
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10 376 **patients are grouped according to whether (closed symbols) or not (open symbols) the**
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12 377 **PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent**
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15 378 **SEM.**
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21 380 **Figure 2. Individual changes from baseline of R_{int} at each dose step, divided according to**
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23 381 **whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁)**
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25 382 **was reached. At each dose step there is large overlap in changes from baseline of R_{int}**
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28 383 **between both groups.**
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31 384
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33 385 **Figure 3. Receiver operator characteristic (ROC) curve showing the sensitivity and false**
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35 386 **positive rate of the change from baseline of R_{int} as a measure to detect a 20% fall in**
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38 387 **FEV₁ (PD₂₀). Area under the curve (AUC) = 0.65.**
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Table 1: Baseline characteristics of children with moderate asthma (n=73) who performed a methacholine provocation test with both R_{int} and FEV_1 measurement at first visit.

		Median (interquartile range)
Age (years)		9.2 (7.6 – 10.6)
Height (cm)		135.6 (128.5 – 146.0)
Sex (number (%) males)		43 (58.9%)
FEV_1 ^a	z-score	-0.26 (-0.74 – 0.32)
	% predicted	96.8 (90.8 – 104.2)
FVC ^b	z-score	0.10 (-0.37 – 0.67)
	% predicted	101.4 (95.7 – 108.3)
FEV_1/FVC	z-score	-0.81 (-1.34 – -0.21)
	% predicted	94.1 (89.7 – 98.6)
R_{int} ^c	z-score	0.78 (0.05 – 1.57)
	% predicted	123.4 (107.0 – 147.2)

a) FEV_1 = Forced expiratory volume in 1 second; b) FVC = Forced expiratory volume; c) R_{int} = Interrupter resistance.

Table 2: Number of children with moderate asthma (n = 73) who reached the PD₂₀ endpoint ^{a)} at subsequent methacholine dose steps.

Dose step	Number
1 = 0.625 µg	0
2 = 1.25 µg	0
3 = 2.5 µg	0
4 = 5.0 µg	2
5 = 10.0 µg	6
6 = 20 µg	10
7 = 40 µg	10
8 = 80 µg	10
9 = 160 µg	9
10 = 320 µg	5
No threshold	21

a) PD₂₀ endpoint = dose step at which a fall of more than 20% in FEV₁ was reached.

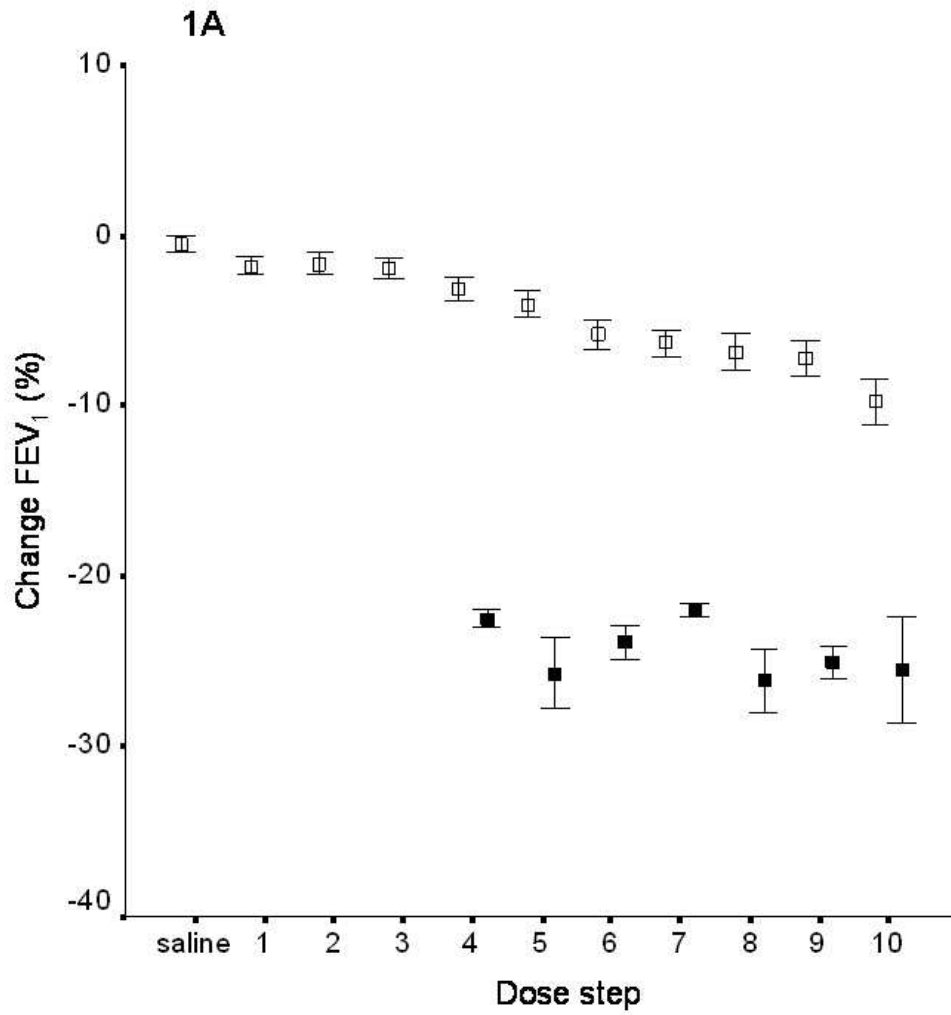


Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent SEM.
179x190mm (96 x 96 DPI)

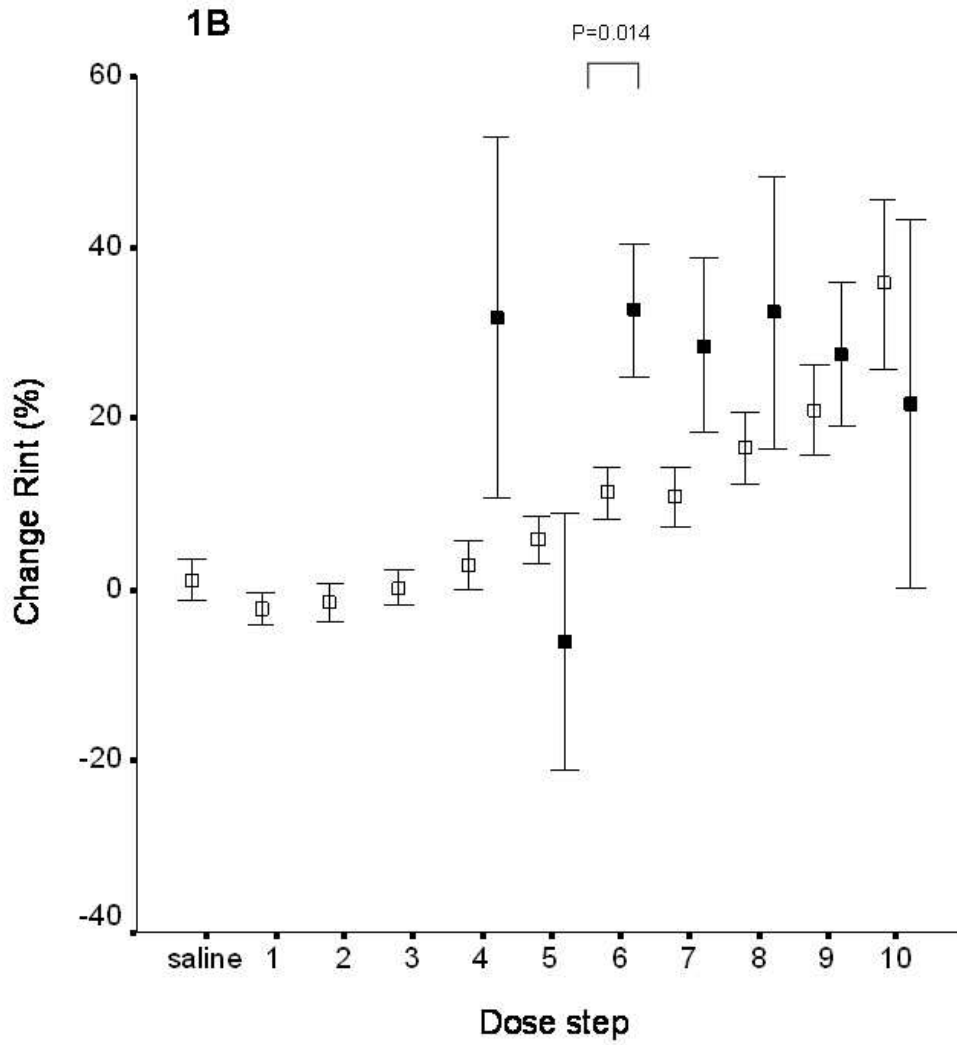


Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent SEM.
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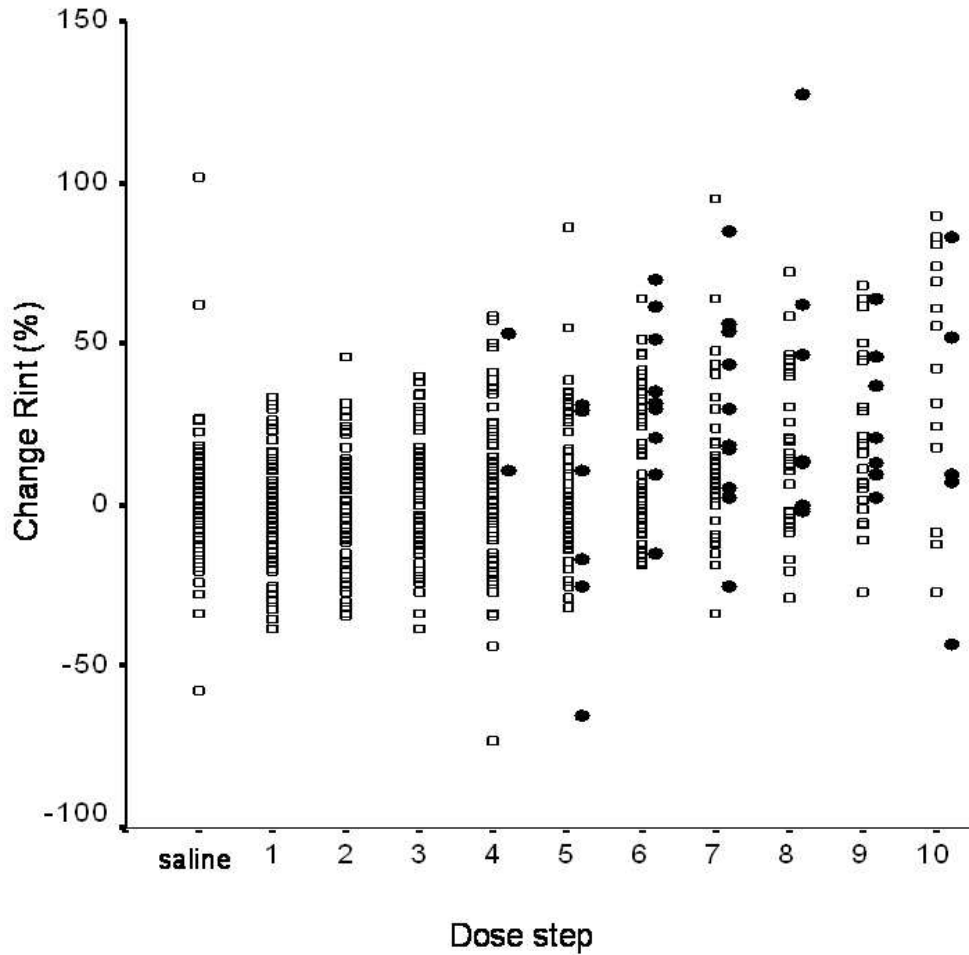


Figure 2. Individual changes from baseline of R_{int} at each dose step, divided according to whether (closed symbols) or not (open symbols) the PD_{20} endpoint (20% fall in FEV_1) was reached. At each dose step there is large overlap in changes from baseline of R_{int} between both groups.
191x190mm (96 x 96 DPI)

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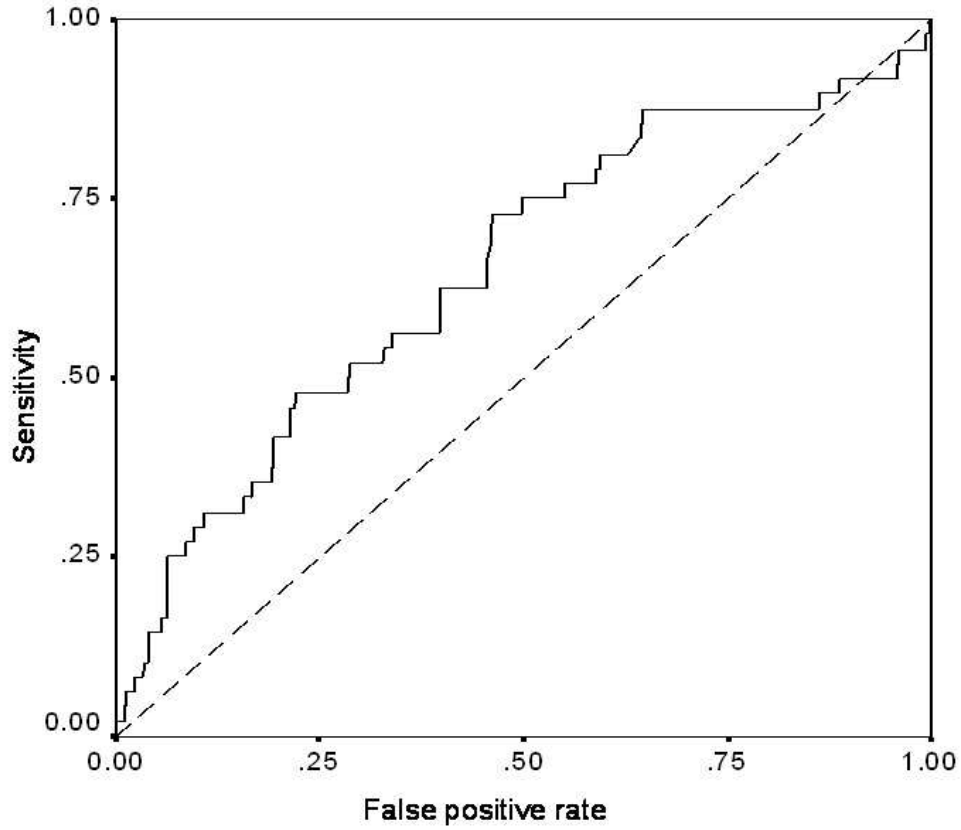


Figure 3. Receiver operator characteristic (ROC) curve showing the sensitivity and false positive rate of the change from baseline of R_{int} as a measure to detect a 20% fall in FEV_1 (PD_{20}). Area under the curve (AUC) = 0.65.
186x161mm (96 x 96 DPI)



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3 1 **ONLINE DATA SUPPLEMENT**
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8 3 EVALUATION OF INTERRUPTER RESISTANCE IN METHACHOLINE CHALLENGE
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10 4 TESTING IN CHILDREN
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15 6 **Authors:** Marije Koopman, MD¹; Hein J.L. Brackel, MD, PhD²; Anja A.P.H. Vaessen-
16
17 7 Verberne, MD, PhD³; Wim C. Hop, PhD⁴; Cornelis K. van der Ent, MD, PhD¹; on behalf of
18
19 8 the COMBO-Rint research group.
20
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22 9
23
24 10 **Institutions:**
25

- 26
27 11 1. Department of Paediatric Pulmonology, Wilhelmina Children's Hospital, University
28
29 12 Medical Centre Utrecht, Utrecht, the Netherlands.
30
31 13 2. Department of Paediatrics, Catharina Hospital, Eindhoven, the Netherlands.
32
33 14 3. Department of Paediatrics, Amphia Hospital, Breda, the Netherlands
34
35 15 4. Department of Biostatistics, Erasmus MC – University Medical
36
37 16 Centre Rotterdam, Rotterdam, the Netherlands
38
39
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41
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43 18 **COMBO-Rint research group consists of:** H.G.M. Arets, N.J. van den Berg, P.L.P. Brand,
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45 19 E.J. Duiverman, H.J. Hendriks, J.W.C.M. Heynens, J.C. van Nierop, M. Nuysink.
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53 22 GlaxoSmithKline Pharma Europe.
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23 MATERIALS AND METHODS

24 This study was a substudy performed in some centres participating in the multicentre
25 COMBO-study. The COMBO-study is a blinded study comparing the clinical effects of
26 fluticasone propionate 200 mcg BD versus salmeterol/fluticasone propionate 50/100 mcg BD
27 on number of symptoms free days in children between 6 and 16 years of age (trial registration
28 number NCT00197106; GSK study number SAM101667) (1). It was performed in 158
29 patients in 19 hospitals in the Netherlands, between June 2005 and October 2008. All children
30 had a clinical history of moderate asthma (according to NAEPP guidelines (2)) and bronchial
31 hyperresponsiveness (BHR) (methacholine dose at which at least 20% fall in FEV₁ (PD₂₀) ≤
32 150 µg). Nine hospitals performed additional R_{int} measurements during methacholine
33 challenge tests, so children from these centres were included in this comparative lung function
34 study. The study was approved by the local medical ethical committees of the different
35 centres and written informed consent was obtained from the parents (and also from the child
36 if ≥12 years old) before the start of the study.

38 Measurements

39 *Methacholine challenge testing*

40 Methacholine challenge tests were performed using the dosimeter method (3;4) with a
41 nebulizer (model 646, DeVilbiss Healthcare, Somerset, USA) attached to a dosimeter
42 (Rosenthal French dosimeter, PDS Instrumentation, Louisville CO, USA). After baseline R_{int}
43 and FEV₁ measurements, 0.9% NaCl was inhaled to rule out non-specific reactions and
44 additionally methacholine chloride was administered in doubling doses (0.625 µg, 1.25 µg,
45 2.5 µg, 5.0 µg, 10.0 µg, 20.0 µg, 40.0 µg, 80 µg, 160 µg, 320 µg). The procedure was
46 performed according to the latest ATS/ERS guidelines (4) as follows: after manually
47 triggering of the dosimeter children were instructed to inhale slowly from forced residual

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3 48 capacity (FRC) to total lung capacity (TLC) and then hold their breath for 2 seconds. Two
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5 49 minutes after each inhalation, R_{int} and FEV_1 were measured. Provocation was continued after
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8 50 at least five minutes and until the PD_{20} or the maximum dose was reached.
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12 52 *Interrupter resistance*

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15 53 Airway resistance measurements were carried out using the MicroRint (Micro Medical
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17 54 Limited, Kent, UK) that contains a pneumotachometer, a flow interruption valve and a
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20 55 pressure transducer to measure mouth pressure post-occlusion. A bacterial filter was used
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22 56 (Spirosafe bacterial filter, Micro Medical, Kent, UK). Measurements were performed conform
23
24 57 the latest ATS/ERS guideline (5). The child was seated and diverted to ensure quiet tidal
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26
27 58 breathing. Children were breathing through a mouth piece while the technicians supported the
28
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30 59 cheeks to reduce the change in upper airway compliance. Ten interruptions of 100 ms were
31
32 60 made at peak expiratory flow with a random frequency, with the valve closing within 10 ms.
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34 61 The MicroRint software automatically rejected measurements that showed an artefact on the
35
36 62 pressure curve. Additionally measurements that showed tachypnoea or irregular breathing,
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39 63 vocalisation, or hyperextension or flexion of the neck were manually discarded as well as
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42 64 were tracings with a horizontal or declining pressure signal suggesting air leakage around the
43
44 65 mouthpiece. R_{int} values were calculated using the two-point linear fit back extrapolation
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46 66 technique to $t=15ms$ (6). A minimum of five successful interruptions was required to calculate
47
48 67 the median R_{int} value. Reference values of Merkus et al. (7) were used to convert R_{int}
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51 68 outcomes into percentage predicted values.
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55 70 *Spirometry*

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57 71 Maximal expiratory flow-volume measurements were performed with standardized
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60 72 equipment. Seven centres used a Jaeger pneumotachometer (Viasys Healthcare, Hochberg,

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3 73 Germany), 1 centre used the Microloop (Micro Medical, Kent, UK) and 1 centre used a Zan
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5 74 spirometer (nSpire Health GmbH, Oberthulba, Germany). Measurements were performed
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7
8 75 according to the latest ATS/ERS guidelines (8) with the child sitting and wearing a nose clip,
9
10 76 without using a bacterial filter. Equipment calibration was performed daily and all
11
12 77 measurements were BTPS corrected. After a full inspiration children performed a maximally
13
14 78 forced and full expiration. At least three technician-accepted flow-volume curves were
15
16 79 obtained and the largest FEV₁ was selected. Reference values of Stanojevic (9) were used.
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22 81 *Analysis; Calculation of inpatient coefficients of variation (CV):*

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24 82 To quantify the variability of FEV₁ and R_{int} measurements between the various dose steps
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26 83 during the methacholine provocation test, a coefficient of variation (CV) was calculated for
27
28 84 each separate patient. This was done by fitting smooth third degree polynomials of each
29
30 85 parameter separately versus log-methacholine dose for each individual patient to allow a
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32 86 gradual change over the subsequent dose steps. The resulting standard deviation of residuals
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34 87 for each patient was subsequently divided by the same patients mean value to obtain an
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36 88 individual CV of the measurements. The resulting individual paired CV's of FEV₁ and R_{int}
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38 89 were compared using Wilcoxon's test.
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45 91 **RESULTS**

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47 92 Visual inspection of individual R_{int} profiles during the challenge test showed large
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49 93 fluctuations with rises and falls at subsequent dose steps. In contrast, much smoother profiles
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51 94 with gradual falls were found for FEV₁ (E-figure 1).
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4 135 **E-FIGURE LEGEND**

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6 136 **E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B)**

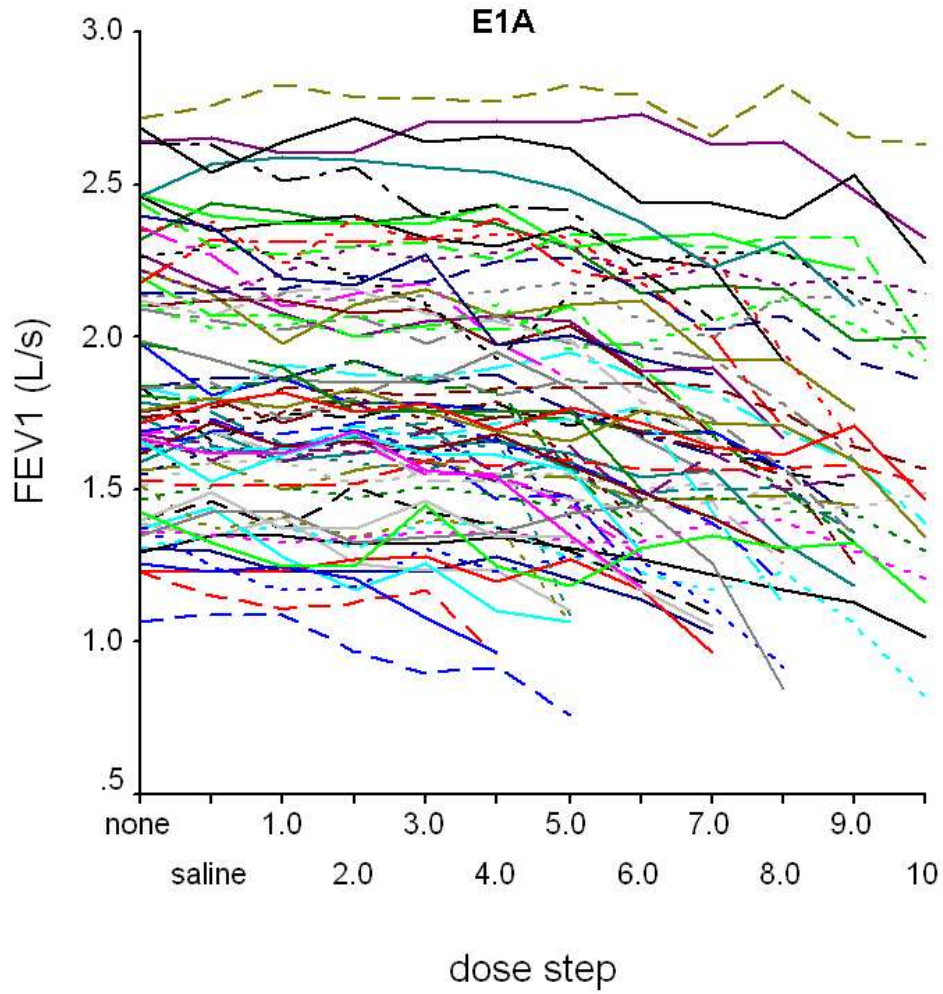
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9 137 **plotted against dose steps of methacholine in 73 children with moderate asthma. Dose**

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11 138 **step 0.5 denotes Saline.**

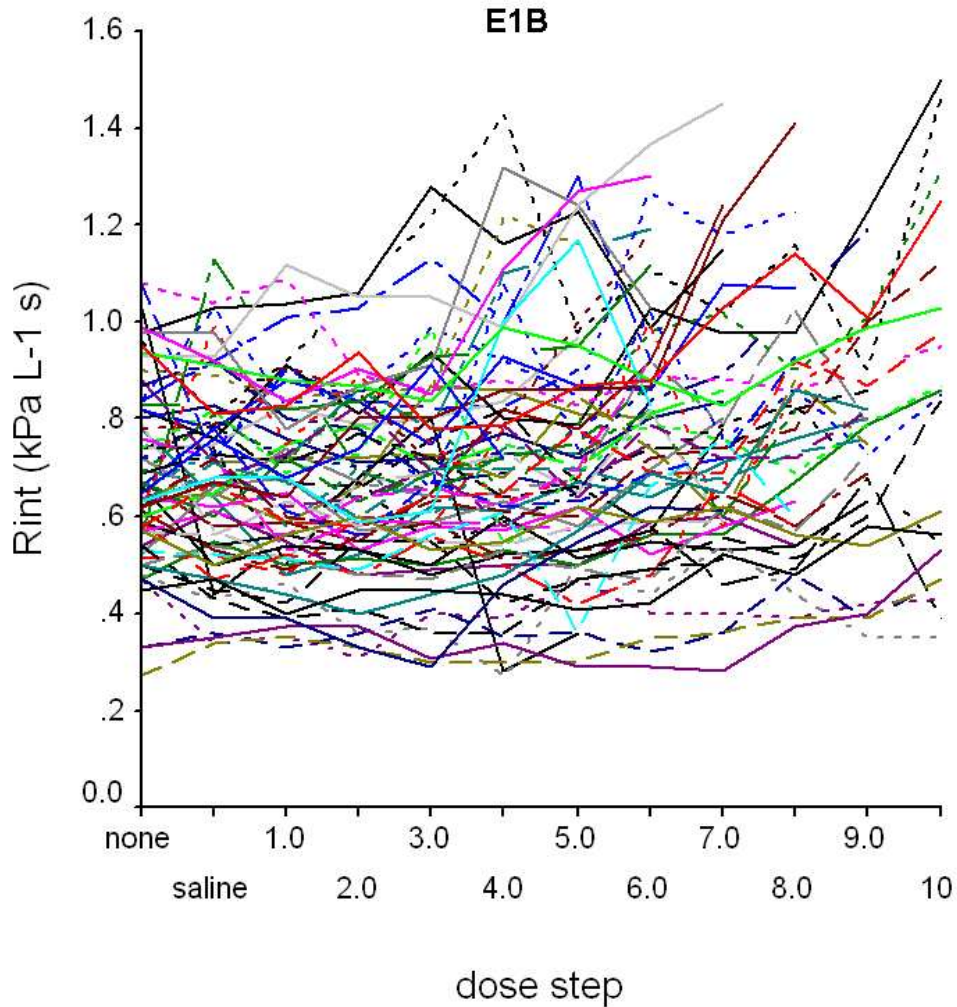
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For Peer Review



E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B) plotted against dose steps of methacholine in 73 children with moderate asthma. Dose step 0.5 denotes Saline. 190x196mm (96 x 96 DPI)



E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B) plotted against dose steps of methacholine in 73 children with moderate asthma. Dose step 0.5 denotes Saline.
190x199mm (96 x 96 DPI)

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3 1 **Title:** Evaluation of interrupter resistance in methacholine challenge testing in children
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14 6

15
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31
32
33
34 14

35
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39
40
41 17

42
43 18 **Corresponding author**

44
45 19 Ms. Marije Koopman

46
47
48 20 Wilhelmina Children's Hospital/University Medical Centre Utrecht

49
50 21 Department of Paediatric Pulmonology, Huispostnummer KH.01.419.0

51
52 22 P O Box 85090, 3508 AB Utrecht, the Netherlands

53
54 23 Email: M.Koopman-5@umcutrecht.nl

55
56 24 Telephone number: 0031-88-7553201 / Fax number: 0031-88-7554747
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20 33 **Running head:** R_{int} in bronchial challenge testing
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3 34 **SUMMARY**
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8 Bronchial hyperresponsiveness is a key feature of asthma and is assessed using bronchial
9 provocation tests. The primary outcome in such tests (a 20% decrease in forced expiratory
10 volume in 1 second (FEV₁)) is difficult to measure in young patients. This study evaluated the
11 sensitivity and specificity of the interrupter resistance (R_{int}) technique, which does not require
12 active patient participation, by comparing it to the primary outcome measure.
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19 41 Methacholine challenge tests were performed in children with a history of moderate
20 asthma and bronchial hyperresponsiveness. Mean and individual changes in R_{int} and FEV₁
21 were studied. A receiver operating characteristic (ROC) curve was used to describe sensitivity
22 and specificity of R_{int}.
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28 45 Seventy-three children (median age: 9.2 yrs; range: 6.3- 13.4 yrs) participated. There
29 was a significant (p< 0.01) increase in mean R_{int} with increasing methacholine doses.
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33 47 However, individual changes of R_{int} showed large fluctuations. There was great overlap in
34 change of R_{int} between children who did and did not reach the FEV₁ endpoint. A ROC-curve
35 showed an area under the curve of 0.65.
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40 50 Because of low sensitivity and specificity, the use of R_{int} to diagnose bronchial
41 hyperresponsiveness in individual patients seems limited.
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47 53 **Word count abstract:** 190
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52 55 **Key words:** Bronchial hyperresponsiveness, bronchial provocation test, forced expiratory
53 volume in 1 second, interrupter resistance, methacholine challenge test, sensitivity,
54 specificity.
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59 INTRODUCTION

60 According to GINA guidelines bronchial hyperresponsiveness (BHR) is a key feature of
61 asthma (1). To objectify BHR, forced flow volume manoeuvres are required to measure the
62 forced expiratory volume in one second (FEV_1), as primary outcome measure in bronchial
63 challenge testing (2). Because of the difficulty of obtaining forced lung function
64 measurements in early childhood the diagnosis of asthma in this age group is challenging and
65 complicated. Several studies have advocated measurement of the interrupter resistance (R_{int})
66 as an alternative to quantify airway obstruction in preschool children and in children with
67 developmental delay (3;4). The interrupter technique is non-invasive and can be obtained
68 during tidal breathing (5). R_{int} can be measured in children from two years of age as it
69 requires minimal cooperation (6). The R_{int} technique has been compared to body
70 plethysmography (7) and transcutaneous partial pressure of oxygen measurements (7-10)
71 during methacholine challenge tests, but most studies made no comparison with the outcome
72 measure FEV_1 . The one study that compared R_{int} with FEV_1 during methacholine challenge
73 testing reported a mean increase in R_{int} with increasing doses of methacholine (11). However,
74 this study did not present the changes in R_{int} for individual patients nor how these related to
75 individual changes in FEV_1 . The ability of R_{int} to detect methacholine induced
76 bronchoconstriction in individual patients, as measured by a 20% decrease in FEV_1 , has never
77 been studied.

78 The latest ATS/ERS statement on pulmonary function testing in preschool children
79 recommended the need to determine the role of R_{int} in bronchial challenge tests (5). The
80 current study evaluated the sensitivity and specificity of R_{int} to detect airflow obstruction
81 during methacholine challenge tests in asthmatic children by comparing measurements with
82 FEV_1 as the gold standard.

83 MATERIALS AND METHODS

84 This study was a substudy of the multicentre COMBO-study in children between 6 and 16
85 years with moderate asthma (see online supplement) (12). Nine out of 19 hospitals performed
86 additional R_{int} measurements during methacholine challenge tests. The children from these
87 centres were included in this substudy. It was approved by the local medical ethical
88 committees and written informed consent was obtained from the parents (and the child if ≥ 12
89 years) before start of the study. For additional information see online supplement.

91 Study protocol

92 Methacholine provocation tests were performed twice (at start of study and after 26 weeks)
93 measuring R_{int} and FEV_1 after each methacholine dose. All children abstained from long-
94 acting β_2 agonists and inhaled steroids for 12 hours before the first methacholine provocation
95 test (and 36 hours before the second methacholine provocation test), and from short acting β_2
96 agonists for 6 hours before provocation testing. To prevent possible, unwanted physiologic
97 effects of deep inhalation on R_{int} , R_{int} was measured prior to FEV_1 . Pulmonary function tests
98 were performed to conform with latest ATS/ERS guidelines (2;5;13) and briefly described
99 below. The complete methodology is described in the online supplement.

101 *Methacholine challenge testing*

102 Methacholine challenge tests were performed using the dosimeter method (2;14) with a
103 nebulizer (model 646, DeVilbiss Healthcare, Somerset, USA) attached to a dosimeter
104 (Rosenthal French dosimeter, PDS Instrumentation, Louisville CO, USA). Methacholine
105 chloride was administered in doubling doses and provocation was continued after at least five
106 minutes and until the dose at which at least 20% fall in FEV_1 (PD_{20}) or the maximum dose
107 was reached.

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109 *Interrupter resistance*

110 Airway resistance measurements were carried out using the MicroRint (Micro Medical
111 Limited, Kent, UK). A bacterial filter was used (Spirosafe bacterial filter, Micro Medical,
112 Kent, UK). Reference values of Merkus et al. (15) were used.

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114 *Spirometry*

115 Maximal expiratory flow-volume measurements were performed with standardized
116 equipment, using reference values of Stanojevic (16).

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118 *Analysis*

119 Changes in FEV₁ and R_{int} were analyzed as percentages change from baseline. Change in R_{int}
120 was also assessed as change in percentage predicted and change in z-score, computed from
121 the latest R_{int} reference values (15). Wilcoxon Signed Ranks test was used to investigate
122 whether there was a significant increase in mean change from baseline of R_{int} at each dose
123 step. To quantify the variability of FEV₁ and R_{int} within individuals between the various
124 dosesteps, coefficients of variation (CV) were calculated (see online supplement for
125 calculation). The resulting individual paired CV's of FEV₁ and R_{int} were compared using
126 Wilcoxon's test. Comparison of mean CV's between centers was done using the Kruskal-
127 Wallis test. The association between changes in FEV₁ and changes in R_{int} at PD₂₀ was
128 analysed using Spearman's correlation (r_s). At each separate dose step Mann Whitney tests
129 were computed to compare the difference in changes from baseline between subjects who did
130 and who did not reach the PD₂₀ endpoint at the dose step. Cox regression, with change from
131 baseline of R_{int} as time-dependent variable, was used to analyse whether these changes were
132 predictive for having reached the PD₂₀ endpoint at the subsequent dose steps. The sensitivity

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3 133 and specificity of R_{int} in relation to having reached FEV₁-based PD₂₀ was described by
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5 134 receiver operating characteristic (ROC) curves. The area under the curve (AUC) shows
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8 135 whether the change in R_{int} can correctly classify those who do and do not reach the PD₂₀
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10 136 endpoint. We used the coefficient of repeatability calculated by Beydon et al. (a 32.1%
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12 137 increase in R_{int}) as a cut-off value to calculate the sensitivity and specificity of R_{int} to predict a
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15 138 20% fall in FEV₁.

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142 RESULTS

143 At the first COMBO-study visit 73 children with moderate asthma (60 % boys) with a median
144 age of 9.2 years (range: 6.3 – 13.4 years) performed both R_{int} and FEV_1 measurements during
145 methacholine challenge. Baseline characteristics are shown in Table 1. Fifty-two children
146 reached the PD_{20} endpoint and were defined as responders (Table 2).

147 There was a significant increase from baseline of R_{int} starting at the sixth methacholine
148 dose step and all dose steps thereafter (all $p < 0.01$). From the first dose step mean FEV_1 was
149 significantly different from baseline value (all $p < 0.01$), except for dose step 2. Visual
150 inspection of individual R_{int} profiles during the challenge test showed large fluctuations with
151 rises and falls at subsequent dose steps. In contrast, much smoother profiles with gradual falls
152 were found for FEV_1 (see E-figure 1 in the online supplement). To express the variability in
153 individual curves, the CV of measured values was calculated per patient. For R_{int} the mean
154 CV of all patients was 10.6% (10th - 90th percentile: 5.5% – 16.3%). The mean CV for FEV_1
155 was significantly smaller: 2.9% (1.7% - 4.3%, $p < 0.001$). Further analysis of CV R_{int} showed
156 that there was no correlation with age ($r_s = -0.13$, $p = 0.26$). Also centres did not significantly
157 differ from each other (Kruskal-Wallis test: $p = 0.17$).

158 Analysing the 52 children who reached the PD_{20} endpoint, there was no significant
159 correlation between changes from baseline of FEV_1 and changes from baseline of R_{int} at the
160 last dose step at which the PD_{20} endpoint was reached ($r_s = 0.11$, $p = 0.44$).

161 Figure 1 demonstrates the mean changes from baseline of FEV_1 and R_{int} at each dose
162 step, while at each dose step patients are grouped according to whether or not the PD_{20}
163 endpoint was reached at the dose step. At none of the dose steps there was a significant
164 difference in the mean change of R_{int} from baseline between those who reached the PD_{20}
165 endpoint at that dose step versus those who did not, except at dose step 6 ($p = 0.014$) (figure
166 1B). A combined analysis of all dose steps comparing responders and non-responders, using

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3 167 Cox regression analysis, showed that a greater change of R_{int} was associated with a higher
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5 168 probability of reaching the PD₂₀ endpoint ($p= 0.038$). However, when inspecting the
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8 169 individual changes from baseline, there is large overlap of children who did and who did not
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10 170 reach the PD₂₀ endpoint at the various dose steps (Figure 2). A ROC curve of changes from
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12 171 baseline of R_{int} as a measure to detect a 20% decrease in FEV₁ is shown in Figure 3, resulting
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15 172 in a small AUC of 0.65.

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17 173 When using a 32.1% increase in R_{int} as cut-off value to calculate the sensitivity and
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19 174 specificity of R_{int} to predict a 20% fall in FEV₁, we found a sensitivity of 50% and a
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22 175 specificity of 43%. This means that R_{int} was able to detect half of the responders as
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24 176 determined by PD₂₀ and that in 12 out of 21 children R_{int} increased with more than 32%
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27 177 without a concomitant fall of FEV₁ with 20% .

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29 178 Repeating all analyses with change in R_{int} expressed as change in percentage predicted
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31 179 and change in z-score led to similar results (data not shown). AUC's of ROC curves for
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34 180 change in percentage predicted and for change in z-score were both 0.66.

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36 181 In 64 children the methacholine challenge test was repeated after 26 weeks. The
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38 182 analyses to compare R_{int} with FEV₁ (as described above) showed similar results (data not
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41 183 shown). The AUC of the ROC curve was 0.61.

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3 186 **DISCUSSION**
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5 187 This study showed that, although there was a significant increase of mean R_{int} values with
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7 188 higher doses of methacholine, individual changes in R_{int} are highly variable during
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10 189 methacholine challenge. The sensitivity and specificity of R_{int} to detect methacholine induced
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12 190 bronchoconstriction is low, which might limit the use of R_{int} in individual patients.
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15 191 Several studies evaluated lung function measures requiring less patient effort than
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17 192 FEV_1 , which could possibly be used as alternative measures of changes in
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20 193 bronchoconstriction. Klug et al showed that R_{int} was significantly less sensitive than specific
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22 194 airway resistance in detecting changes in airway function (7). Phagoo et al found that R_{int} was
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24 195 also less sensitive and showed more variability than transcutaneous partial pressure of oxygen
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26 196 measurements (9;10). In a study of Beydon et al. the majority of children with a 20% fall in
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28 197 transcutaneous partial pressure of oxygen ($PD_{20}P_{tc}O_2$) showed a significant change of R_{int}
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30 198 during methacholine challenge. However, all children who did not reach the $PD_{20}P_{tc}O_2$ had at
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32 199 least once a significant change from baseline (8), resulting in many false positive as well as
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35 200 false negative outcomes.
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38 201 Despite these data R_{int} is still advocated as an alternative measure for airway
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40 202 obstruction in those who are not able to perform forced breathing manoeuvres. Several studies
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42 203 showed significant correlation between gold standard FEV_1 and R_{int} in groups of patients
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44 204 (4;6), and reference values for R_{int} measurement in healthy controls have been published
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46 205 (15;17-20). Although there is convincing evidence on the value of R_{int} measurement as a
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48 206 measure of airway obstruction in groups of patients, the sensitivity of R_{int} to detect changes in
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50 207 airway obstruction in individual patients has been little researched. Only one previous study
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52 208 compared R_{int} with FEV_1 during methacholine challenge testing (11) and found that on
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54 209 average R_{int} increased during methacholine challenge comparable to changes in FEV_1
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56 210 measurements, but individual changes in R_{int} were not shown. Kannisto et al. compared R_{int}
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3 211 with FEV₁ during exercise challenge to diagnose BHR (21). However, in this study only 11
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5 212 (22%) children reached the R_{int} endpoint (15% increase from baseline) and only 6 of them
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8 213 also reached the FEV₁ endpoint (10% decrease from baseline). The children who showed a
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10 214 15% rise in R_{int} but who did not have a 10% decrease in FEV₁ could be misclassified by R_{int}
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12 215 as having BHR. Davies et al. investigated the correlation between R_{int} and FEV₁ in
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15 216 bronchodilator response, and despite a correlation between FEV₁ and R_{int} in a group of
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17 217 patients, they found no significant correlation in individual children (22).

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20 218 To evaluate the sensitivity and specificity of the R_{int} technique to detect changes in
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22 219 airway obstruction in individual children we performed simultaneous measurements of R_{int}
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24 220 and gold standard FEV₁ in a large group of children with asthma. We show that in individual
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26 221 cases the variability of R_{int} measurements is high (mean intra-patient CV 10.6%), precluding
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28 222 proper prediction of changes in FEV₁. In the present study the ROC curve, describing the
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30 223 sensitivity and specificity of R_{int} in detecting BHR as determined by the primary outcome
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32 224 FEV₁, had a small AUC of 0.65. This predictive capacity is lower compared to impulse
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34 225 oscillometry (AUC 0.68 to 0.75 (23)).

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38 226 Large individual fluctuations in R_{int} might be due to large inpatient short-term
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40 227 variation in airway resistance during tidal breathing (11;17;20;24). This variability, resulting
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42 228 in low sensitivity and specificity, could limit the ability to detect thresholds in individual
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44 229 airway obstruction as required in clinical practice. Furthermore the sensitivity might be lower
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46 230 compared to FEV₁ because during (severe) airflow obstruction equilibration of alveolar
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48 231 pressure might take longer than is realised during the shutter closure time during R_{int}
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50 232 measurement (25). This may explain the lower R_{int} values in case of (severe) peripheral
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52 233 airway obstruction and hence lead to misclassifications during methacholine challenge
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54 234 testing. A third explanation of high variability of R_{int} might be the variability of upper airway
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3 235 resistance. Proper positioning of the head and neck and adequate support of the cheeks is
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5 236 highly warranted and might lead to intra-individual variability.
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8 237 The findings of our study imply that on group level BHR can be documented, since we
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10 238 showed on average a significant rise in R_{int} with increasing methacholine doses. This may be
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12 239 useful in epidemiologic research. However, R_{int} seems to have insufficient capacity to
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14 240 diagnose BHR in individual patients and therefore it is of limited value in BHR measurements
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16 241 in clinical practice.
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19 242 A major strength of our study is the fact that the methacholine challenge test was
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21 243 repeated after 26 weeks and resulted in a similarly low sensitivity and specificity of R_{int} ,
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23 244 which validated the results. Beside this, the present study may have some limitations. Firstly,
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25 245 the study was performed in children with a mean age of 9.3 years, because preschool children
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27 246 are seldom able to perform FEV_1 measurements. It could be questioned if the found results
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29 247 are applicable to younger children. Since R_{int} is a measure of total airway resistance, an
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31 248 induced bronchoconstriction will lead to a relative larger increase in R_{int} in young children
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33 249 compared to older children, because in young children lower airways resistance contributes
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35 250 more to total airway resistance (26). Nevertheless, the fluctuations in R_{int} are expected to be
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37 251 similar as the short term repeatability of R_{int} that has been found to be independent of age
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39 252 (20;27). Additionally we used a 20% fall as 'gold standard' in detecting methacholine induced
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41 253 bronchoconstriction. When we used a 32% increase in R_{int} (the coefficient of repeatability of
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43 254 R_{int} measurements as described by Beydon et al.) as a cut-off value to determine BHR, there
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45 255 were 12 children with a significant change in R_{int} but without a 20% fall in FEV_1 . The
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47 256 pathophysiology of a fall in FEV_1 during a forced manoeuvre might be different from an
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49 257 increase of R_{int} during tidal breathing. Therefore, there is a possibility that FEV_1 might not be
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51 258 the gold standard for bronchial challenges especially in young children.
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3 259 The current study showed on average an increase of R_{int} with subsequent methacholine
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6 260 doses during methacholine challenge tests and therefore it might be useful as a measure of
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8 261 airflow obstruction in groups of patients, e.g. in large epidemiological studies. However, we
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10 262 conclude that R_{int} seems to have insufficient capacity to diagnose BHR in individual patients.
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3 369 **FIGURE LEGENDS**
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8 371 **Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step**
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10 372 **patients are grouped according to whether (closed symbols) or not (open symbols) the**
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12 373 **PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent**
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15 374 **SEM.**
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21 376 **Figure 2. Individual changes from baseline of R_{int} at each dose step, divided according to**
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23 377 **whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁)**
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25 378 **was reached. At each dose step there is large overlap in changes from baseline of R_{int}**
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28 379 **between both groups.**
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33 381 **Figure 3. Receiver operator characteristic (ROC) curve showing the sensitivity and false**
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35 382 **positive rate of the change from baseline of R_{int} as a measure to detect a 20% fall in**
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37 383 **FEV₁ (PD₂₀). Area under the curve (AUC) = 0.65.**
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Table 1: Baseline characteristics of children with moderate asthma (n=73) who performed a methacholine provocation test with both R_{int} and FEV_1 measurement at first visit.

		Median (interquartile range)
Age (years)		9.2 (7.6 – 10.6)
Height (cm)		135.6 (128.5 – 146.0)
Sex (number (%) males)		43 (58.9%)
FEV_1 ^a	z-score	-0.26 (-0.74 – 0.32)
	% predicted	96.8 (90.8 – 104.2)
FVC ^b	z-score	0.10 (-0.37 – 0.67)
	% predicted	101.4 (95.7 – 108.3)
FEV_1/FVC	z-score	-0.81 (-1.34 – -0.21)
	% predicted	94.1 (89.7 – 98.6)
R_{int} ^c	z-score	0.78 (0.05 – 1.57)
	% predicted	123.4 (107.0 – 147.2)

a) FEV_1 = Forced expiratory volume in 1 second; b) FVC = Forced expiratory volume; c) R_{int} = Interrupter resistance.

Table 2: Number of children with moderate asthma (n = 73) who reached the PD₂₀ endpoint ^{a)} at subsequent methacholine dose steps.

Dose step	Number
1 = 0.625 µg	0
2 = 1.25 µg	0
3 = 2.5 µg	0
4 = 5.0 µg	2
5 = 10.0 µg	6
6 = 20 µg	10
7 = 40 µg	10
8 = 80 µg	10
9 = 160 µg	9
10 = 320 µg	5
No threshold	21

a) PD₂₀ endpoint = dose step at which a fall of more than 20% in FEV₁ was reached.

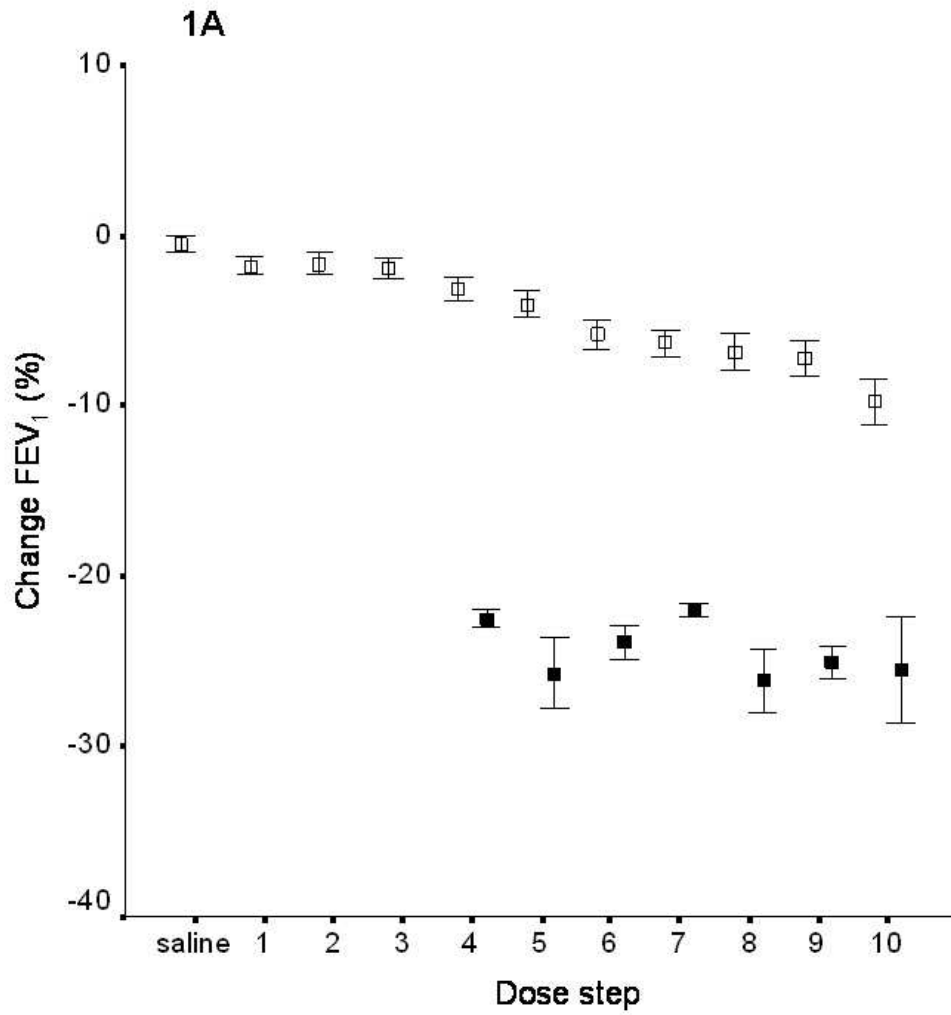


Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent SEM.
179x190mm (96 x 96 DPI)

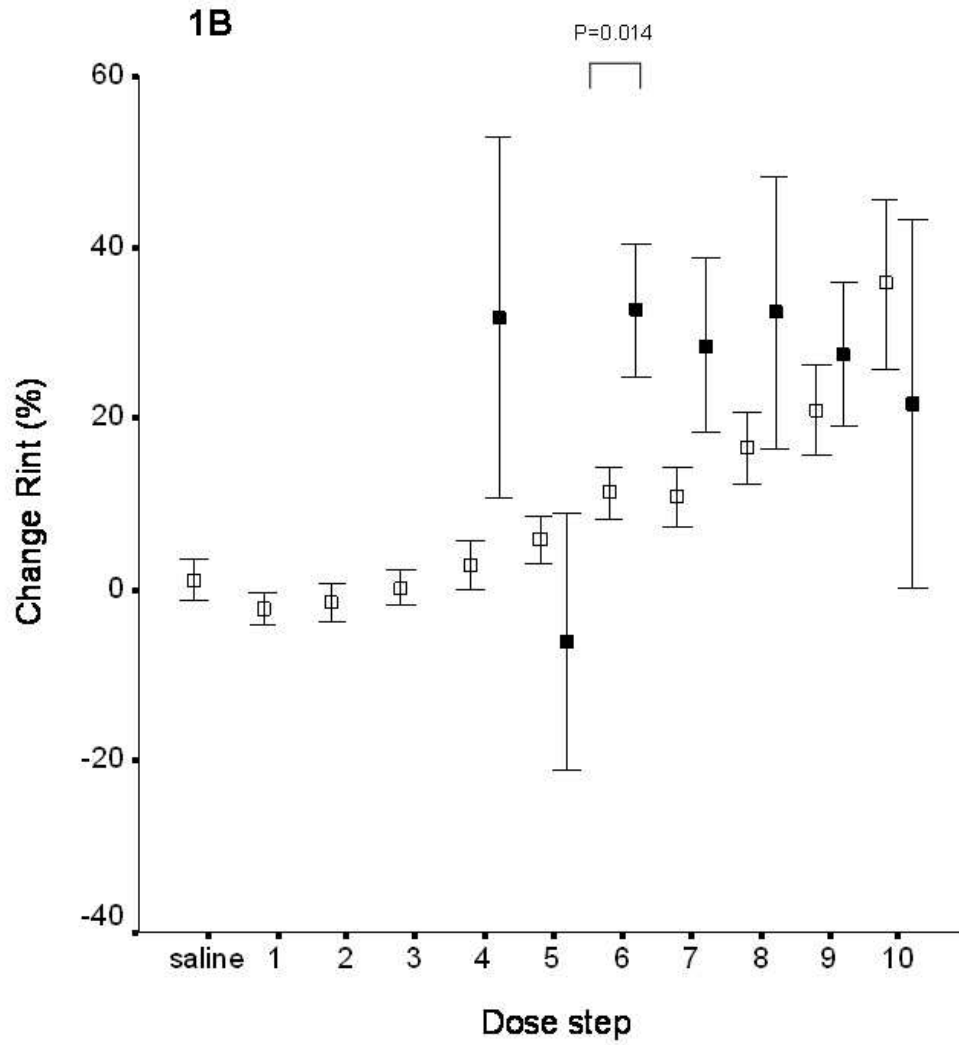


Figure 1. Mean changes from baseline of FEV₁ (1A) and R_{int} (1B). At each dose step patients are grouped according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached at that dose step. Error bars represent SEM.
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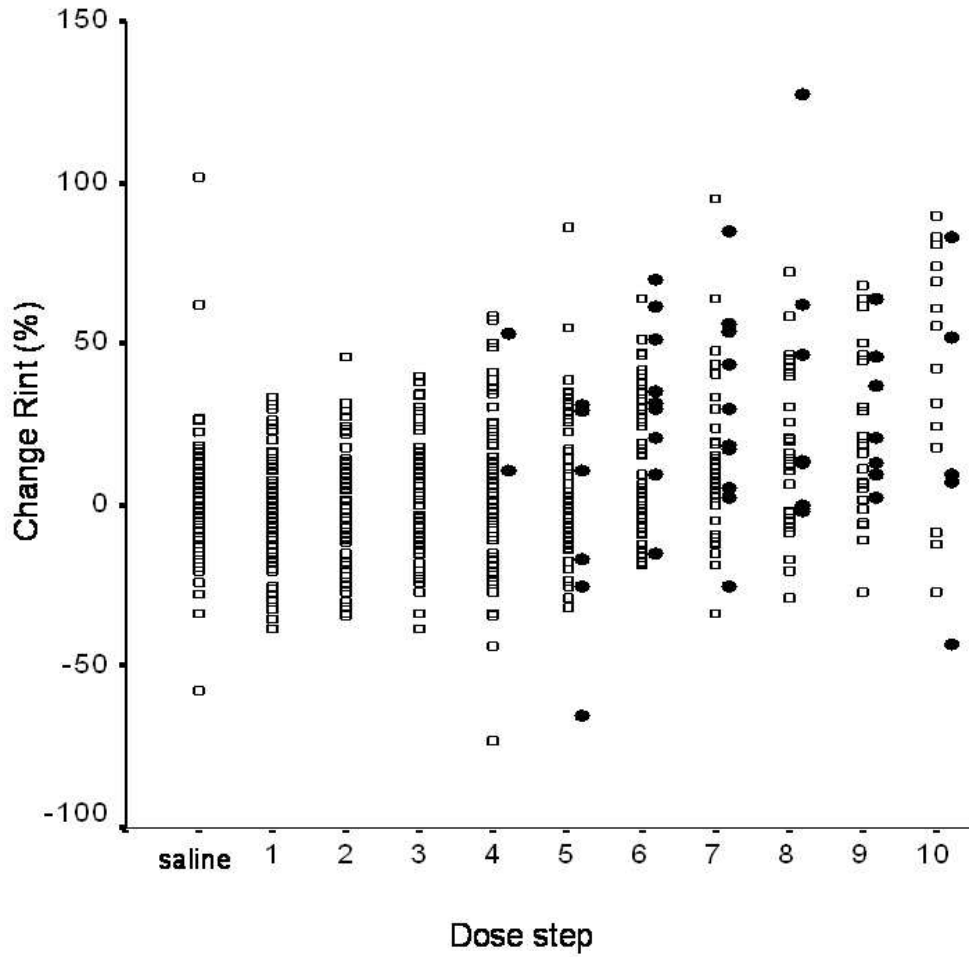


Figure 2. Individual changes from baseline of R_{int} at each dose step, divided according to whether (closed symbols) or not (open symbols) the PD₂₀ endpoint (20% fall in FEV₁) was reached. At each dose step there is large overlap in changes from baseline of R_{int} between both groups.
191x190mm (96 x 96 DPI)

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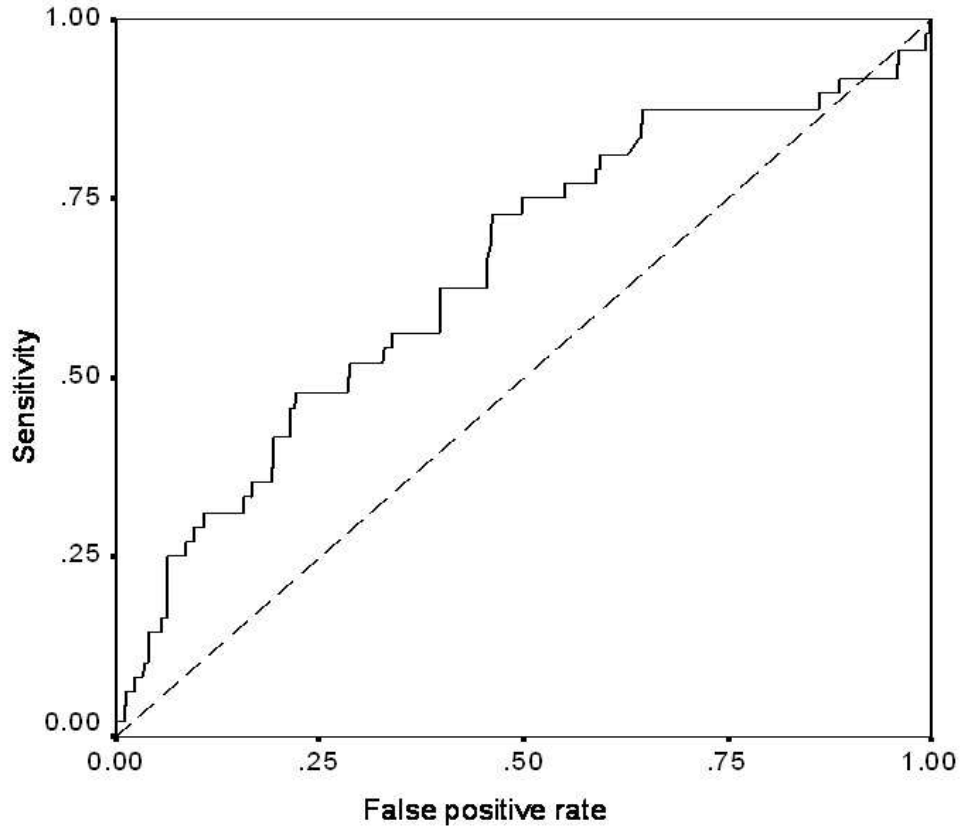


Figure 3. Receiver operator characteristic (ROC) curve showing the sensitivity and false positive rate of the change from baseline of R_{int} as a measure to detect a 20% fall in FEV_1 (PD_{20}). Area under the curve (AUC) = 0.65.
186x161mm (96 x 96 DPI)



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3 **1 ONLINE DATA SUPPLEMENT**
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8 3 EVALUATION OF INTERRUPTER RESISTANCE IN METHACHOLINE CHALLENGE
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10 4 TESTING IN CHILDREN
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15 6 **Authors:** Marije Koopman, MD¹; Hein J.L. Brackel, MD, PhD²; Anja A.P.H. Vaessen-
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17 7 Verberne, MD, PhD³; Wim C. Hop, PhD⁴; Cornelis K. van der Ent, MD, PhD¹; on behalf of
18
19 8 the COMBO-Rint research group.
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22 9
23
24 10 **Institutions:**
25

- 26
27 11 1. Department of Paediatric Pulmonology, Wilhelmina Children's Hospital, University
28
29 12 Medical Centre Utrecht, Utrecht, the Netherlands.
30
31 13 2. Department of Paediatrics, Catharina Hospital, Eindhoven, the Netherlands.
32
33 14 3. Department of Paediatrics, Amphia Hospital, Breda, the Netherlands
34
35 15 4. Department of Biostatistics, Erasmus MC – University Medical
36
37 16 Centre Rotterdam, Rotterdam, the Netherlands
38
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41
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43 18 **COMBO-Rint research group consists of:** H.G.M. Arets, N.J. van den Berg, P.L.P. Brand,
44
45 19 E.J. Duiverman, H.J. Hendriks, J.W.C.M. Heynens, J.C. van Nierop, M. Nuysink.
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53 22 GlaxoSmithKline Pharma Europe.
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23 MATERIALS AND METHODS

24 This study was a substudy performed in some centres participating in the multicentre
25 COMBO-study. The COMBO-study is a blinded study comparing the clinical effects of
26 fluticasone propionate 200 mcg BD versus salmeterol/fluticasone propionate 50/100 mcg BD
27 on number of symptoms free days in children between 6 and 16 years of age (trial registration
28 number NCT00197106; GSK study number SAM101667) (1). It was performed in 158
29 patients in 19 hospitals in the Netherlands, between June 2005 and October 2008. All children
30 had a clinical history of moderate asthma (according to NAEPP guidelines (2)) and bronchial
31 hyperresponsiveness (BHR) (methacholine dose at which at least 20% fall in FEV₁ (PD₂₀) ≤
32 150 µg). Nine hospitals performed additional R_{int} measurements during methacholine
33 challenge tests, so children from these centres were included in this comparative lung function
34 study. The study was approved by the local medical ethical committees of the different
35 centres and written informed consent was obtained from the parents (and also from the child
36 if ≥12 years old) before the start of the study.

38 Measurements

39 *Methacholine challenge testing*

40 Methacholine challenge tests were performed using the dosimeter method (3;4) with a
41 nebulizer (model 646, DeVilbiss Healthcare, Somerset, USA) attached to a dosimeter
42 (Rosenthal French dosimeter, PDS Instrumentation, Louisville CO, USA). After baseline R_{int}
43 and FEV₁ measurements, 0.9% NaCl was inhaled to rule out non-specific reactions and
44 additionally methacholine chloride was administered in doubling doses (0.625 µg, 1.25 µg,
45 2.5 µg, 5.0 µg, 10.0 µg, 20.0 µg, 40.0 µg, 80 µg, 160 µg, 320 µg). The procedure was
46 performed according to the latest ATS/ERS guidelines (4) as follows: after manually
47 triggering of the dosimeter children were instructed to inhale slowly from forced residual

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3 48 capacity (FRC) to total lung capacity (TLC) and then hold their breath for 2 seconds. Two
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5 49 minutes after each inhalation, R_{int} and FEV_1 were measured. Provocation was continued after
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8 50 at least five minutes and until the PD_{20} or the maximum dose was reached.
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12 52 *Interrupter resistance*

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15 53 Airway resistance measurements were carried out using the MicroRint (Micro Medical
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17 54 Limited, Kent, UK) that contains a pneumotachometer, a flow interruption valve and a
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20 55 pressure transducer to measure mouth pressure post-occlusion. A bacterial filter was used
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22 56 (Spirosafe bacterial filter, Micro Medical, Kent, UK). Measurements were performed conform
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24 57 the latest ATS/ERS guideline (5). The child was seated and diverted to ensure quiet tidal
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27 58 breathing. Children were breathing through a mouth piece while the technicians supported the
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29 59 cheeks to reduce the change in upper airway compliance. Ten interruptions of 100 ms were
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31 60 made at peak expiratory flow with a random frequency, with the valve closing within 10 ms.
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34 61 The MicroRint software automatically rejected measurements that showed an artefact on the
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36 62 pressure curve. Additionally measurements that showed tachypnoea or irregular breathing,
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38 63 vocalisation, or hyperextension or flexion of the neck were manually discarded as well as
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41 64 were tracings with a horizontal or declining pressure signal suggesting air leakage around the
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43 65 mouthpiece. R_{int} values were calculated using the two-point linear fit back extrapolation
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45 66 technique to $t=15ms$ (6). A minimum of five successful interruptions was required to calculate
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48 67 the median R_{int} value. Reference values of Merkus et al. (7) were used to convert R_{int}
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50 68 outcomes into percentage predicted values.
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55 70 *Spirometry*

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57 71 Maximal expiratory flow-volume measurements were performed with standardized
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60 72 equipment. Seven centres used a Jaeger pneumotachometer (Viasys Healthcare, Hochberg,

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3 73 Germany), 1 centre used the Microloop (Micro Medical, Kent, UK) and 1 centre used a Zan
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5 74 spirometer (nSpire Health GmbH, Oberthulba, Germany). Measurements were performed
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8 75 according to the latest ATS/ERS guidelines (8) with the child sitting and wearing a nose clip,
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10 76 without using a bacterial filter. Equipment calibration was performed daily and all
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12 77 measurements were BTPS corrected. After a full inspiration children performed a maximally
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14 78 forced and full expiration. At least three technician-accepted flow-volume curves were
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16 79 obtained and the largest FEV₁ was selected. Reference values of Stanojevic (9) were used.
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22 81 *Analysis; Calculation of inpatient coefficients of variation (CV):*

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24 82 To quantify the variability of FEV₁ and R_{int} measurements between the various dose steps
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26 83 during the methacholine provocation test, a coefficient of variation (CV) was calculated for
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28 84 each separate patient. This was done by fitting smooth third degree polynomials of each
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30 85 parameter separately versus log-methacholine dose for each individual patient to allow a
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32 86 gradual change over the subsequent dose steps. The resulting standard deviation of residuals
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34 87 for each patient was subsequently divided by the same patients mean value to obtain an
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36 88 individual CV of the measurements. The resulting individual paired CV's of FEV₁ and R_{int}
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38 89 were compared using Wilcoxon's test.
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45 91 **RESULTS**

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47 92 Visual inspection of individual R_{int} profiles during the challenge test showed large
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49 93 fluctuations with rises and falls at subsequent dose steps. In contrast, much smoother profiles
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51 94 with gradual falls were found for FEV₁ (E-figure 1).
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4 135 **E-FIGURE LEGEND**

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6 136 **E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B)**

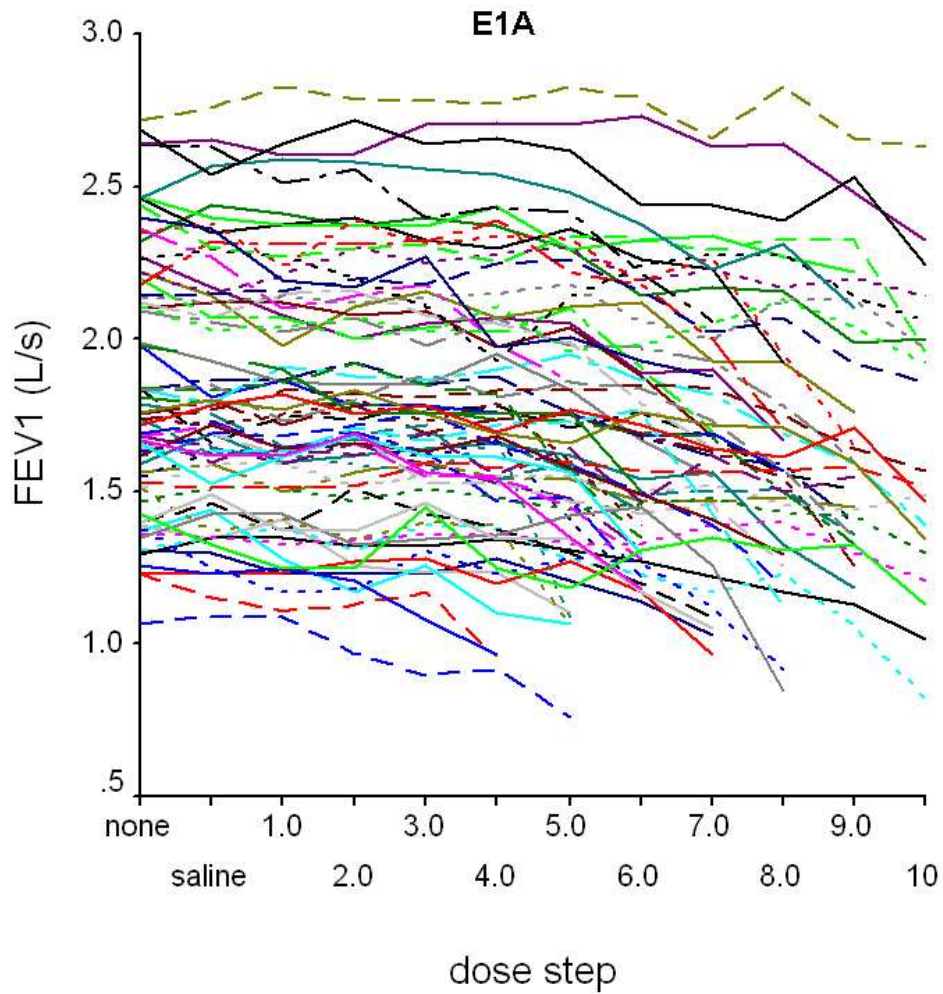
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9 137 **plotted against dose steps of methacholine in 73 children with moderate asthma. Dose**

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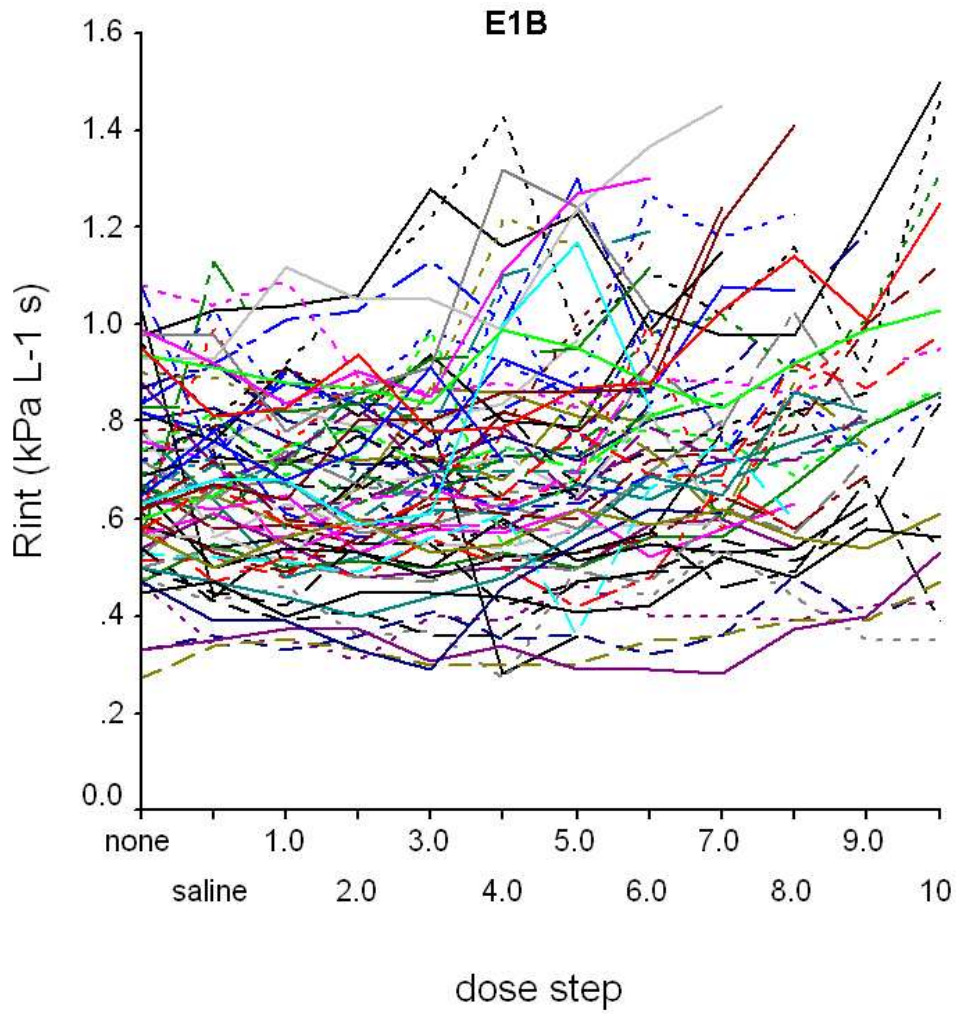
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For Peer Review



E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B) plotted against dose steps of methacholine in 73 children with moderate asthma. Dose step 0.5 denotes Saline. 190x196mm (96 x 96 DPI)

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E-figure 1. Individual fluctuations of absolute values of FEV₁ (E1A) and R_{int} (E1B) plotted against dose steps of methacholine in 73 children with moderate asthma. Dose step 0.5 denotes Saline. 190x199mm (96 x 96 DPI)