Differences in LCI and FRC between two commercial Multiple Breath Washout devices in healthy children and adults.

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Online supplementary materials:

Methods:

This online supplement contains detailed descriptions of randomisation, in vitro protocol, study population, software versions, in vivo protocol, power calculation and statistical methods, as well as supplementary table S1 and figures S1 and S2.

Randomisation

MBNW tests were performed in random order as determined by a computer-generated randomisation sequence in vitro (using the lung model) and in vivo using the two different devices. For the in vivo measurements, a second randomisation took place to determine the order of the breathing protocol, either relaxed or fixed tidal breathing. Cross-over testing for both randomisations took place during the second visit, with an interval of at least 1 day.

In vitro protocol

The appropriate dead space reducer set was used for the different target FRC on the EM. To avoid jet stream during measurements with ndd a filter with a dead space of 38 ml was used. BTPS correction was based on one time calibration with an external unit per test for the EM.

After at least 5 stable breaths given by the ventilator, the washout phase was started by a switch to 100% oxygen. The test ended when the end tidal Nitrogen (N₂) concentration had decreased to < 1/40th of the start concentration for at least 3 consecutive breaths. Between measurements, a waiting time of at least the duration of a test was used to expel any residual oxygen within the lung model and to ensure the N₂ had returned to baseline level.

Recruitment and inclusion criteria

Healthy children and adults were recruited from the hospital (UMCG), social networks of the researchers, advertisements in schools and from volunteers who took part in the NORM study (clinicaltrials.gov: NCT00848406), which is a study to obtain reference values in healthy adult subjects.

Health was defined as current non- (passive) smoker with < 0.5 pack years of (passive) smoking history, no history of birth below 36 weeks of gestational age, no history of lower respiratory tract or cardiac disease, no use of inhalation medication within the last year, no history of acute respiratory illness in the last 6 weeks and no current reported pregnancy.
Written informed consent was obtained from the caregivers, the caregivers and volunteer, or the volunteers only according to age.

In vivo protocol

MBNW was performed in a seated position with a nose clip on. After at least 5 stable breaths, the washout phase was started by switching to 100% oxygen and was continued until the N₂ concentration in the exhaled air was reduced to ≤ 1/40th of the starting concentration. FRC was calculated from the net volume of N₂ expired, divided by the difference between end-tidal N₂ at the start and end of the washout, following the ERS/ATS statement ¹. LCI was calculated as the cumulative expired volume (CEV) divided by the corresponding FRC.

All MBNW tests underwent visual quality control by a trained researcher (AZ) before analysis. Tests were excluded if there was evidence of leaks, unacceptable irregular breathing or if the tidal volume during fixed tidal breathing was out of the mandatory range. At least two technical acceptable MBNW tests with a difference in FRC and LCI below 10% between the tests were required. Outcome parameters were calculated as the mean of all included tests in the final analysis.

Software versions

Software versions 1.9.0.19 of the ndd and 3.1.6 of the EM were used for the in vitro tests. These tests were recalculated using the newest software versions 3.02.00.06 (07-2017) and 3.2, respectively. The software version 2.01.00.09 (03-2016) of the ndd was used for the in vivo measurements which included the new N₂ algorithm. Tests were reloaded using software version 3.02.00.06 (07-2017) in which FRC calculation was optimized. For the EM, in vivo measurement the software version 3.2 was used.

Both devices calculate the N₂ concentration indirectly. The EM uses Dalton’s law to compute N₂ by subtraction of CO₂ and O₂ in expired air. In the used ndd software version for this study N₂ is computed by a combination of Dalton’s law and molar mass measurement on a point by point basis for the entire expirogram.

Power calculation:

To achieve 80% power with a two-sided significance level (α) of 0.05 to detect a difference of 10% in LCI at least 9 healthy adults needed to be included, based on the data of Raaijmakers et al. ²⁰. We
anticipated that inclusion of a wider range (and variance) of FRC measurements, as well as inclusion of children, together with an estimated drop-out rate of 10% of randomised subjects, required a final enrollment of 20 adults and 20 children.

Statistical analysis:
Within-test repeatability of LCI was expressed as the intra individual coefficient of variation (CV%) that was defined as (SD/Mean) *100 of at least 2 single washout maneuvers within one test occasion.

Demographic variables such as age, gender, height, weight, and BMI were expressed as means with range for continuous variables, and number (percentages) for dichotomous variables. Spirometric parameters were expressed as z-scores according to the Global Lung Initiative reference values 29.

Differences between groups were analyzed by student’s t-test for normally distributed variables and Mann-Whitney U test for non-normal distributed variables.

Post hoc analysis
EM calculates both FRC as LCI at the airway opening, whereas ndd calculates FRC at the airway opening and CEV at the gas sampling point. To exclude the influence of dead space correction on LCI between the two devices a post hoc analysis was performed to re-calculate LCI\textsubscript{ndd} in LCI at airway opening (LCI\textsubscript{ao-ndd}). This option became available in the latest software version (version V3.05.01.07, 03-2019).

Results

Table S1: Difference in outcome parameters between fixed and relaxed tidal breathing

<table>
<thead>
<tr>
<th></th>
<th>ndd</th>
<th></th>
<th></th>
<th>EM</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Absolute</td>
<td>P</td>
<td>Absolute</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>FRC (l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child</td>
<td>0.07</td>
<td>(-0.22; 0.08)</td>
<td>0.33</td>
<td>0.01</td>
<td>(-0.17; 3.9)</td>
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<tr>
<td>Adult</td>
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<td>(-0.29; 0.21)</td>
<td>0.74</td>
<td>-0.14</td>
<td>(-0.38; 0.10)</td>
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<td>LCI</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Child</td>
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<td>(-0.4;0.3)</td>
<td>0.81</td>
<td>0.02</td>
<td>(-0.3; 0.4)</td>
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<td>0.37</td>
<td>-0.3</td>
<td>(-0.6; 0.0)</td>
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<tr>
<td>CEV (l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child</td>
<td>-0.5</td>
<td>(-1.5; 0.5)</td>
<td>0.34</td>
<td>0.4</td>
<td>(-1.3; 2.0)</td>
</tr>
<tr>
<td></td>
<td>VT (ml)</td>
<td>VT/kg (ml)</td>
<td>RR (/min)</td>
<td></td>
<td></td>
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<td>-------</td>
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<td></td>
</tr>
<tr>
<td>Child</td>
<td>-9 (-61; 43)</td>
<td>0.73 (-3.0; 1.4)</td>
<td>-1.0 (-3.4; 1.3)</td>
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<td></td>
</tr>
<tr>
<td>Adult</td>
<td>246 (100; 391)</td>
<td>0.00 (3.8; 6.6)</td>
<td>-1.1 (-2.3; 0.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Figure S1: Agreement of FRC: Bland-Altman plots of FRC measurements of a) Ndd (EasyOne Pro Lab) and body plethysmography (pleth), b) Ndd and helium dilution technique (He), c) EM (Exhalyzer D) and pleth and d) EM and He. Data are plotted as relative differences (in %). The continuous line represents the mean difference, the fine dashed lines the upper and lower limits of agreement (mean difference ± 1.96 SD) and the rough dashed lines the defined limits of clinical acceptability (5 to -10% for comparison to pleth, and 10 to -10% for comparison to He). Open circles represent children; closed circles adults.
Figure S2: Bland-Altman plots of agreement between relaxed (RTB) and fixed tidal breathing (FTB) a) FRC (functional residual capacity) on ndd (EasyOne Pro Lab) and b) LCI (lung clearance index) on ndd, c) FRC on EM (Exhalyzer D) and d) LCI on EM. Data are plotted as relative differences (in %). The continuous line represents the mean difference, the fine dashed lines the upper and lower limits of agreement (mean difference ± 1.96 SD) and the rough dashed lines the defined limits of clinical acceptability (10 to -10%). Open circles represent children; closed circles adults...