Normative data for multiple breath washout outcomes in school-aged Caucasian children

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ONLINE SUPPLEMENT

METHODS

MBW measurements

Each subject was tested in a single visit and performed at least two trials. During the test the child sat in an upright position wearing a nose-clip and was asked to breath regularly through a snorkel-like mouthpiece connected to a bacterial filter (Air Safety Eco Slimline, No. 4222/01), spirette, and dead space reducer (set 2 for subjects ≤35 kg, set 3 for subjects >35 kg). The washout was stopped following at least three tidal breaths below 1/40th of the pre-phase end-tidal N_2 concentration [13].

MBW analysis

The original temperature and pressure conditions were applied to the data and the software corrected appropriately for equipment-related dead space. The pre-capillary dead space was 33.3 ml for all measurements collected with both set 2 and set 3 [29]. The post-capillary dead space was either 9.5 ml for set 2 (children up to 35kg) or 22 ml for set 3 (children greater than 35kg).

Differences in the analysis between Spiroware 3.1.6 and Spiroware 3.2.1

The open-circuit hardware we used for N_2MBW (Exhalyzer D, Eco Medics AG, Duernten, Switzerland) measures F_{N2} indirectly based on Dalton's law [E1]. A side-stream oxygen (O_2) sensor and a mainstream carbon dioxide (CO_2) sensor measure gas concentrations. These two signals must be aligned in order to allow the calculation of the N_2 signal. A static synchronization method has been used in previous software versions including Spiroware version 3.1.6 [E1]. During a measurement, flow, O_2, and CO_2 signals are not stable throughout the measurement and depend on the breathing pattern of the subject. Spiroware version 3.2.1 constantly calculates new delay times based on the breathing pattern (dynamic delay correction, DDC) [E2].

Apart from the different synchronization method, the calculation method of the re-inspired N_2 volume differs also between the two software versions. In Spiroware 3.1.6 this is calculated from the post-capillary dead space (number of breaths x post-capillary dead space) [E3], while Spiroware 3.2.1 uses the integral between inspiratory flow and N_2 concentration to calculate it [E4].

Reloading measurements in Spiroware 3.2.1

To ensure that our data were analyzed using the new DDC synchronization method we reloaded the raw data (recorded in Spiroware 3.1.6) into Spiroware 3.2.1. First, we performed a new synchronization of the signals by reloading the raw data (A-files) from one measurement per subject into the channel synchronization tool in Spiroware. Five consecutive breaths of good quality from the pre-washout phase were used to generate new static delay times. Each trial was then reloaded manually into Spiroware 3.2.1 to ensure calculation of the DDC was applied. The quality of the signal alignment was checked by visual control. The original temperature and pressure conditions were applied to the data and the software corrected appropriately for equipment-related dead space.

Quality control of MBW trials

The following criteria were used to assess quality of the entire dataset of MBW measurements after reloading in SPW 3.2.1: no evidence of a leak, stable pre-washout phase, regular breathing pattern during the washout, and at least three consecutive breaths with the end tidal concentration of N_2 below 2.5% of the pre-washout phase N_2 concentration[E3]. In addition, trials with a drift in end tidal CO_2 concentration out of the range of 4-6% across the washout were excluded[E5]. Tests with at least two technically acceptable trials with FRC values within 25% of the mean were included for analysis.

RESULTS

FRC equation

The full regression equation for FRC is given below. The standard deviation of the residuals for the model was 0.1632.

$$\text{Ln FRC} = -18.18016 + 3.98197*\ln(\text{height}) - 0.31707*\ln(\text{weight})$$

Univariate Analysis	Coefficient	95% Confidence interval	p-value
$LCI_{2.5\%}$			
Height (cm)	-0.0053	-0.0087; -0.0019	**0.002**
Weight (kg)	-0.0049	-0.0090; -0.0008	**0.020**
Age (y)	-0.0225	-0.0421; -0.0030	**0.024**
Vd/V_T (%)	0.0258	0.0093; 0.0422	**0.002**
V_T/FRC (%)	0.0040	-0.0013; 0.0093	0.135
Sex	0.1413	-0.0113; 0.2712	0.133
$LCI_{5\%}$			
Height (cm)	-0.0046	-0.0070; -0.0023	**<0.001**
Weight (kg)	-0.0040	-0.0069; -0.0011	**0.007**
Age (y)	-0.0178	-0.0316; -0.0039	**0.012**
Vd/V_T (%)	0.0160	0.0042; 0.0278	**0.008**
V_T/FRC (%)	0.0073	0.0037; 0.0109	**<0.001**
Sex	0.0867	-0.0058; 0.1796	0.066
M_1/M_0			
Height (cm)	-0.0009	-0.0016; -0.0002	**0.009**
Weight (kg)	-0.0008	-0.0016; 0.0002	0.060
Age (y)	-0.0030	-0.0069; 0.0009	0.133
Vd/V_T (%)	0.0050	0.0017; 0.0083	**0.003**
V_T/FRC (%)	0.0010	-0.0001; 0.0020	0.069
Sex	0.0274	-0.0015; 0.0532	0.382
M_2/M_0			
Height (cm)	-0.0068	-0.0114; -0.0023	**0.003**
Weight (kg)	-0.0058	-0.0114; -0.0003	**0.040**
Age (y)	-0.0249	-0.0514; 0.0016	0.065
Vd/V_T (%)	0.0161	-0.0066; 0.03871	0.164
V_T/FRC (%)	0.0147	0.0079; 0.0215	**<0.001**
Sex	0.1475	-0.02858; 0.3236	0.100
Ln FRC (L)			
Ln Height (cm)	3.0997	2.9136; 3.286	**<0.001**
Ln Weight (kg)	0.9668	0.8778; 1.0558	**<0.001**
Ln Age (y)	1.1836	1.0857; 1.2814	**<0.001**
Ln Vd/V_T (%)	-0.6429	-0.8042; -0.4815	**<0.001**
Ln V_T (L)	0.6918	0.5694; 0.8142	**<0.001**
Sex	-0.0270	-0.1580; 0.1039	0.684

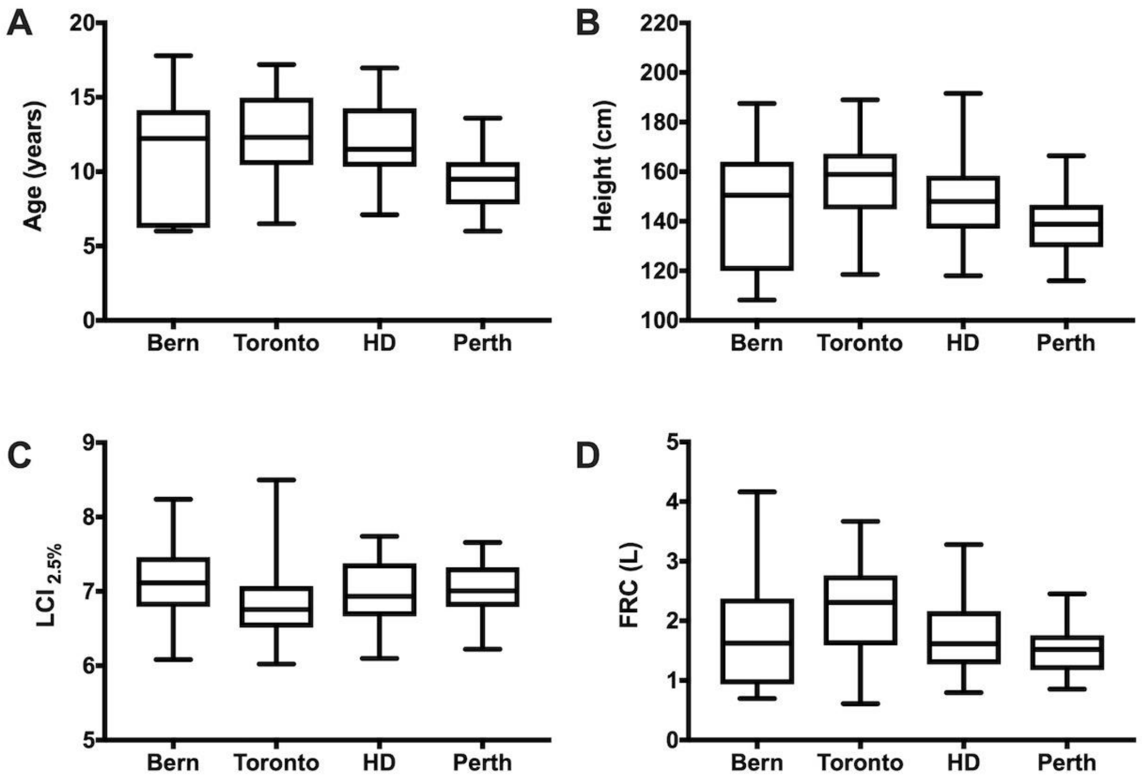
Supplemental Table S1: Univariate linear regression model describing the association between lung clearance index at 2.5% ($LCI_{2.5\%}$) and 5% ($LCI_{5\%}$), moment ratios 1 (M_1/M_0) and 2 (M_2/M_0), and functional residual capacity (FRC) with demographic and physiological parameters. V_T/FRC (%): tidal volume/functional residual capacity, Vd/V_T (%): dead space volume / tidal volume.

Multivariate Analysis	Coefficient	95% Confidence interval	p-value
$LCI_{2.5\%}$			
Age (y)	0.04	0.01; 0.07	0.02
Vd/V_T (%)	0.07	0.04; 0.10	<0.001
V_T/FRC (%)	0.02	0.01; 0.02	<0.001
$LCI_{5\%}$			
Age (y)	0.04	0.02; 0.06	<0.001
Vd/V_T (%)	0.07	0.05; 0.08	<0.001
V_T/FRC (%)	0.02	0.01; 0.02	<0.001
M_1/M_0			
Age (y)	0.01	0.01; 0.02	<0.001
Vd/V_T (%)	0.02	0.01; 0.02	<0.001
V_T/FRC (%)	0.004	0.00; 0.005	<0.001
M_2/M_0			
Age (y)	0.07	0.03; 0.10	<0.01
Vd/V_T (%)	0.10	0.06; 0.14	<0.001
V_T/FRC (%)	0.03	0.02; 0.04	<0.001
Ln FRC (L)			
ln Height (cm)	3.98	3.43; 4.54	<0.001
ln Weight (kg)	-0.32	-0.51; -0.13	<0.01

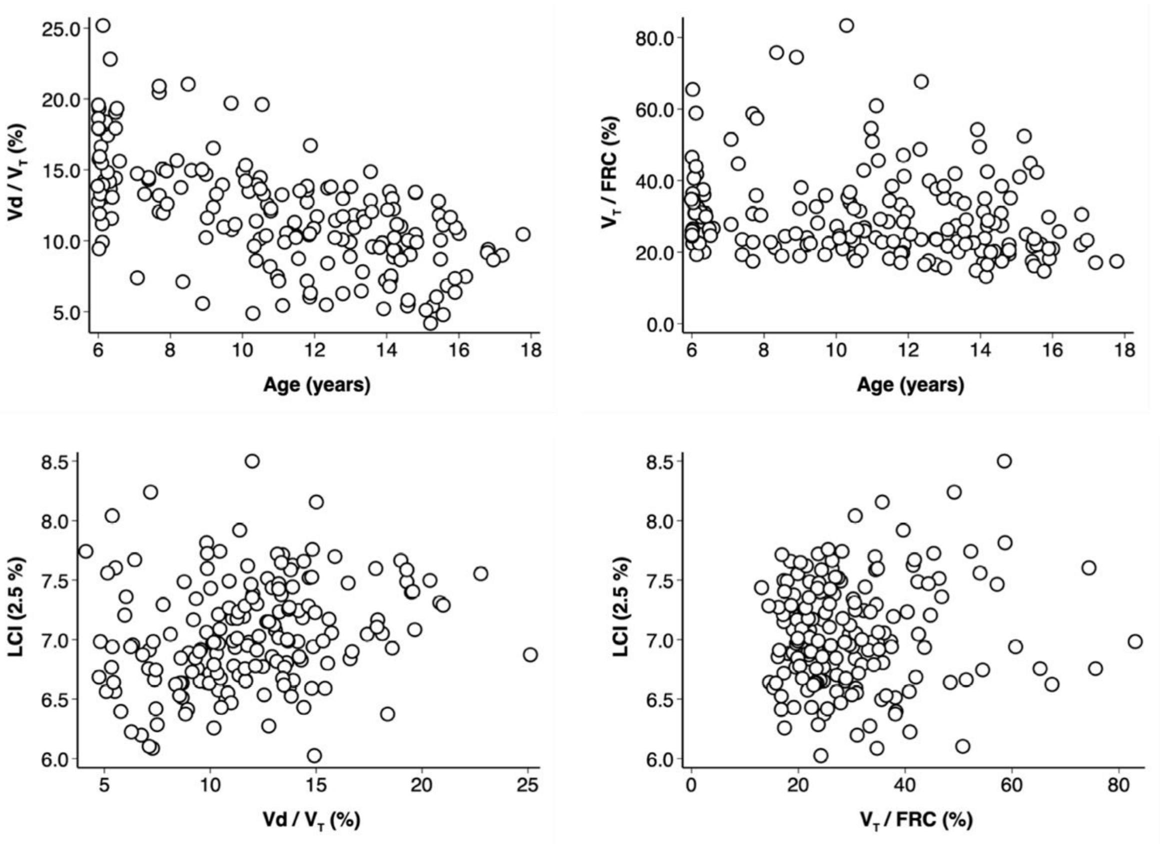
Supplemental Table S2: Final multiple linear regression model describing the association between lung clearance index at 2.5% ($LCI_{2.5\%}$) and 5% ($LCI_{5\%}$), moment ratios 1 (M_1/M_0) and 2 (M_2/M_0), and functional residual capacity (FRC) with demographic and physiological parameters. V_T/FRC (%): tidal volume/functional residual capacity, Vd/V_T (%): dead space volume / tidal volume.

Figures

Supplemental Figure S1: Distribution of age, height, lung clearance index at 2.5% ($LCI_{2.5\%}$) and functional residual capacity (FRC) per study center. HD indicates Heidelberg.



Supplemental Figure S2: Association of Vd/V_T (%) and V_T/FRC (%) with age, and association of LCI 2.5% with Vd/V_T (%) and V_T/FRC (%) in 180 healthy children.



References

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