ONLINE SUPPLEMENT

Elevated Lung Clearance Index in infants with cystic fibrosis shortly after birth

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SUPPLEMENTAL METHODS

Statistical analysis

We compared the relationship between lung volume (tidal volume and FRC) and body size separately in CF and healthy infants in a post-hoc approach using linear regression analysis. In order to compare the regression models between the two groups, we repeated the regression analysis in all children by adding an interaction term between body size and the groups. The regression coefficient for this interaction term then corresponds to the difference between the slope of CF and healthy infants, and the evidence for an interaction was assessed using the Wald p-value for the interaction term.

SUPPLEMENTAL RESULTS

Study population

Healthy infants were all term-born (*Fuchs O. et al., Int J Epidemiol 2012*). Three infants with CF were mildly preterm with 36.0, 36.1 and 36.6 weeks of gestation, respectively. These infants did not require medical support at birth. Infants with CF showed significantly lower birth weight compared to healthy infants. After adjustment for gestational age, this difference was not significant anymore. Three infants with CF were included despite an incomplete wash-out curve (SF_6 end-expiratory-concentration ranging from 2.5% to 2.9% at the end of the test).

We found no age dependency of LCI and age in our healthy infants, (β -0.19, 95% CI -0.38 – 0.01, p = 0.067, Figure S9). Therefore we used one single ULN value derived from our healthy study population.

Multiple-breath wash-out measurements in WBreath (main analysis)

Intra-test variability for the 37 infants with CF and 34 healthy infants with three valid tests per child was similar for LCI with a mean CV of 5.9% (SD ± 4.2%) in CF compared to 5.2% (SD ± 3.2%) in healthy children, while mean CV of FRC was 7.0% (SD ± 6.4%) in CF compared to 8.9% (SD ± 5.4%) in healthy infants (both not significantly different).

There is one patient with CF with an outstandingly elevated LCI of 11.94 representing a true physiological outlier and therefore included in the final analysis. Additional results of the comparison of LCI and FRC between healthy infants and infants with CF without this outlier are presented in Table S3. Mean (SD) LCI 6.98 (0.68) was elevated in infants with CF compared to 6.78 (± 0.43) in healthy controls. The resulting mean difference of 0.21 (95% CI

0.01 – 0.42, p = 0.059) was lower compared to the analysis including this outlier (0.30, 95% CI 0.02 – 0.58, p = 0.034).

Sensitivity analysis

(i) Wash-in measurements in WBreath

In 4 infants with CF one of the wash-in trials was included in analysis despite incomplete wash-in (end-concentration ranging from 2.6 to 2.9% at the end of the test). We compared 53 valid wash-in measurements of infants with CF to 56 wash-in measurements of healthy controls and found a mean difference for LCI of 0.38 (95% CI 0.07 – 0.68, p = 0.015, Table S4, Figure S1). Thereof, 9 (17%) out of 53 infants with CF showed an LCI above the ULN. FRC was also significantly elevated in infants with CF (mean difference 17.7 ml, 95% CI 11.1 – 24.4 ml, p < 0.001, Figure S2). Eighteen infants with CF (34 %) presented FRC values above the ULN, and 4 infants with CF (8 %) presented both elevated values for LCI and FRC.

(iia) Wash-out measurements in LungSim

We compared 53 wash-out measurements of infants with CF to 57 measurements of healthy controls analysed with the alternative software (LungSim 2.1.0, NM GmbH, Thalwil, Switzerland) (*Anagnostopoulou P. et al., Physiol Meas 2016*). Mean difference was 0.56 (95% CI 0.18 – 0.94, p = 0.005, Table S4, Figure S3). Thereof, 13 (23%) out of 53 infants with CF showed an LCI above the ULN. FRC was also significantly elevated in infants with CF (mean difference 10.5 ml, 95% CI 5.1 – 15.9 ml, p < 0.001, Figure S4). Sixteen infants with CF (30 %) presented FRC values above the ULN, and 3 (6 %) infants with CF presented both elevated values for LCI and FRC.

(iib) Wash-in measurements in LungSim

We compared 52 wash-in measurements of infants with CF to 56 measurements of healthy controls analysed with the alternative software LungSim. Mean difference was 0.87 (95% CI 0.47 – 1.27, $p < 0.001$, Table S4). Thereof, 21 (40%) out of 52 infants with CF showed an LCI above the ULN. FRC was also significantly elevated in infants with CF (mean difference 10.2 ml, 95% CI 4.6 – 15.9 ml, $p < 0.001$). Fifteen infants with CF (29%) presented FRC values above the ULN, and 4 (8 %) infants with CF presented both elevated values for LCI and FRC.

(iii) Children with at least two valid wash-out measurements in WBreath

At least two valid MBW tests were obtained in 47 (89%) infants with CF and 56 (98%) healthy infants. Mean LCI 7.12 (± 1.00 SD) in infants with CF was significantly higher than in healthy infants with 6.79 (± 0.42) (mean difference 0.33, 95% CI 0.04 – 0.63, $p = 0.025$, Figure S5). Also mean FRC 126.9 ml (± 20.9 ml) in infants with CF was significantly higher compared to 112.2 ml (± 15.6 ml) in healthy infants (mean difference 14.8 ml, 95% CI 7.6 – 21.9 ml), $p < 0.001$), Figure S6).

Regulatory mechanism of breathing

Comparison of the regression models between CF and healthy by adding an interaction term (of weight and FRC, and weight and tidal volume respectively) showed a statistically borderline significant difference between the slope in CF and healthy for the correlation of weight with FRC ($\beta = 0.01$, 95% CI -0.00003 – 0.02, $p = 0.051$, Figure 4), while there was no significant difference for the slope between the two groups for the correlation of weight with tidal volume (Figure S8).

SUPPLEMENTAL TABLES

Table S1. Number of successful and unsuccessful multiple-breath wash-out trials and reasons for exclusions.

	All (n = 110)	**Healthy (n = 57)**	**CF (n = 53)**
Total number of trials	494	278	216
Successful trials	284	147	137
Infants with 3 successful trials (%)	71 (65%)	34 (60%)	37 (70%)
Infants with 2 successful trials (%)	32 (29%)	22 (39%)	10 (19%)
Infants with 1 successful trials (%)	7 (6%)	1 (1%)	6 (11%)
Unsuccessful trials due to	210	131	79
leak	19	14	5
sigh	140	67	73
arousal	21	7	14
movement	54	33	21
other reason	69	24	45

The table shows the total number of trials, number of successful and unsuccessful trials and reasons for trial exclusion. Often several reasons led to the exclusion of a trial, therefore the sum of individual reasons of exclusion exceeds the number of unsuccessful trials.

Table S2. Centre's affiliation of CF infants.

CF centre	CF infants (n=53)
Bern	17
Zürich	15
Lausanne	6
Aarau	5
Basel	3
Geneva	3
Ticino	3
St. Gallen	1

Results are displayed in total numbers.

Table S3. Comparison of wash-out parameters excluding one true physiological CF outlier.

	Healthy	CF	Mean difference (95% CI)	p-value
LCI	6.78 ± 0.43	7.08 ± 0.96	0.30 (0.02 – 0.58)	0.034
LCI		*6.98 ± 0.68*	*0.21 (0.01 – 0.42)*	*0.059*
FRC (ml)	112.3 ± 15.5	126.7 ± 20.3	14.5 (7.7 – 21.3)	< 0.001
FRC (ml)		*125.5 ± 18.4*	*13.3 (6.8 – 19.7)*	*< 0.001*
FRC per body weight (ml /kg)	25.4 ± 3.9	28.3 ± 4.3	2.9 (1.3 – 4.4)	< 0.001
FRC per body weight (ml /kg)		*28.3 ± 4.3*	*2.9 (1.4 – 4.5)*	*< 0.001*

Data are given as mean ± SD and compared by Student's *t* test.

LCI: Lung clearance index; FRC: Functional residual capacity**.**

The first line shows the final results of the included washout-out measurements of infants with CF (n = 53) and healthy infants (n = 57) in the standard software WBreath. The second line (*in italic*) presents results excluding one patient with CF with an outstandingly elevated LCI value of 11.94.

Table S4. Wash-out and wash-in parameters in infants with CF and healthy infants using two different softwares.

	Healthy	**CF**	**Mean difference (95% CI)**	**p-value**
WBreath 3.28.0				
Multiple-breath wash-in	*n = 56*	*n = 53*		
LCI	6.65 ± 0.51	7.03 ± 1.02	0.38 (0.07 – 0.68)	0.015
FRC (ml)	108.9 ± 14.9	126.6 ± 20.0	17.7 (11.1 – 24.4)	< 0.001
FRC per body weight (ml/kg)	24.6 ± 3.6	28.3 ± 4.6	3.7 (2.2 – 5.3)	< 0.001
Multiple-breath wash-out	*n = 57*	*n = 53*		
LCI	6.78 ± 0.43	7.08 ± 0.96	0.30 (0.02 – 0.58)	0.034
FRC (ml)	112.3 ± 15.5	126.7 ± 20.3	14.5 (7.7 – 21.3)	< 0.001
FRC per body weight (ml/kg)	25.4 ± 3.9	28.3 ± 4.3	2.9 (1.3 – 4.4)	< 0.001
LungSim 2.1.0				
Multiple-breath wash-in	*n = 56*	*n = 52*		
LCI	6.84 ± 0.62	7.71 ± 1.36	0.87 (0.47 – 1.27)	< 0.001
FRC (ml)	103.3 ± 13.0	113.5 ± 16.6	10.2 (4.6 – 15.9)	< 0.001

FRC per body weight (ml/kg)	23.3 ± 3.3	25.4 ± 4.1	2.1 (0.6 – 3.5)	0.005
Multiple-breath wash-out	*n = 57*	*n = 53*		
LCI	6.93 ± 0.67	7.49 ± 1.29	0.56 (0.18 – 0.94)	0.005
FRC (ml)	99.8 ± 12.2	110.2 ± 22.4	10.5 (5.1 – 15.9)	< 0.001
FRC per body weight (ml/kg)	22.6 ± 3.3	24.6 ± 3.9	2.1 (0.7 – 3.4)	0.003

Data are given as mean ± SD and compared by Student's *t* test.

LCI: Lung clearance index; FRC: Functional residual capacity.

Table S5. Association between lung clearance index and other lung function parameters.

	Univariable model			Multivariable model*		
LCI	β	95% CI	p-value	β	95% CI	p-value
Functional residual capacity (ml)	0.012	0.005 – 0.019	< 0.001	0.008	-0.001 – 0.016	0.036
Tidal volume (ml/kg)	0.023	-0.002 – 0.048	0.07			
Respiratory rate (/min)	0.012	-0.004 – 0.028	0.13			
Peak insp. flow (ml/sec)	0.021	0.009 – 0.034	0.001			
Peak exp. flow (ml/sec)	0.014	0.003 – 0.024	0.011	0.011	0.001 – 0.021	0.033
tPTEF/tE	-0.020	-0.032 – -0.007	0.003	-0.014	-0.026 – -0.001	0.035

Results from the linear regression models assessing the association between lung function indices averaged from 100 tidal breaths and lung clearance index from subsequent multiple-breath wash-out. Univariable and multivariable regression models for LCI. In the multivariable regression model only functional residual capacity, peak expiratory flow and tPTEF/tE remained significantly associated with LCI (R^2 0.19). *Adjusted for age, body size and sex. Per unit change in a given lung function index, LCI changes by the effect size of the regression coefficient β.

LCI: Lung clearance index; tPTEF/tE: Time to peak tidal expiratory flow (*t*PTEF)/expiratory time (tE) ratio.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. **Lung clearance index of wash-in measurements analysed in the standard software WBreath.** Lung clearance index (LCI) and group means of sulfur hexafluoride multiple-breath wash-in in 56 healthy infants and 53 infants with cystic fibrosis (CF). Analysis were performed in the standard software WBreath. The dashed line represents the upper limit of normal (mean healthy + 1.64*SD healthy).

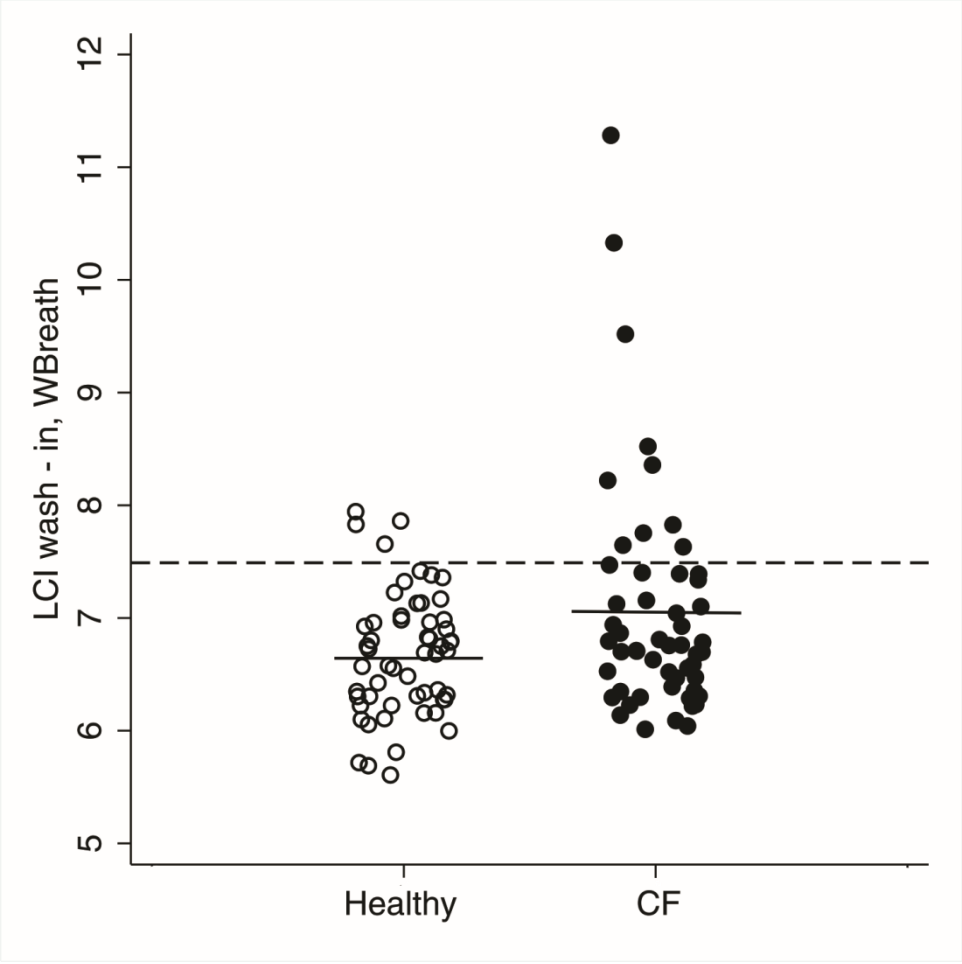

Figure S2. **Functional residual capacity of wash-in measurements analysed in the standard software WBreath.** Functional residual capacity (FRC) and group means of sulfur hexafluoride multiple-breath wash-in in 56 healthy infants and 53 infants with cystic fibrosis (CF). Analysis were performed in the standard software WBreath. The dashed line represents the upper limit of normal (mean healthy + 1.64*SD healthy).

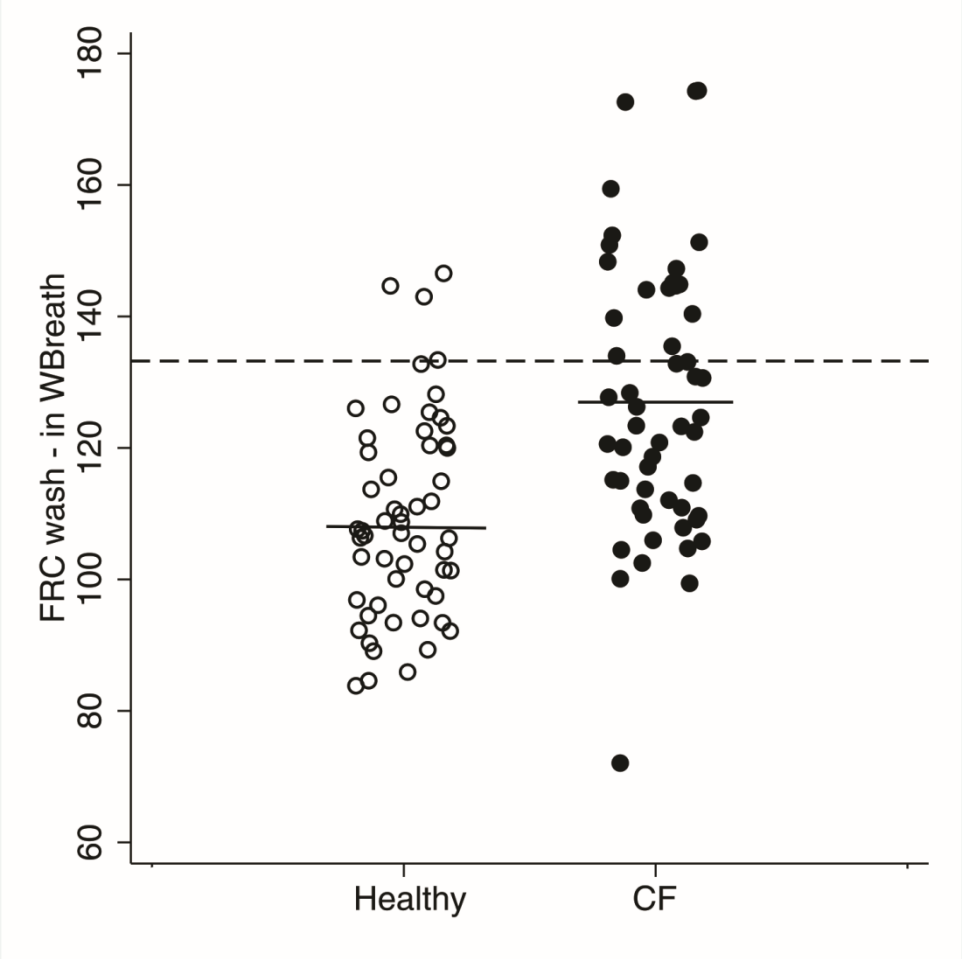

Figure S3. **Lung clearance index of wash-out measurements analysed in the alternative software LungSim.** Lung clearance index (LCI) and group means of sulfur hexafluoride multiple-breath wash-out in 57 healthy infants and 53 infants with cystic fibrosis (CF). Analysis were performed in the alternative software (LungSim 2.1.0, NM GmbH, Thalwil, Switzerland). The dashed line represents the upper limit of normal (mean healthy + 1.64*SD healthy).

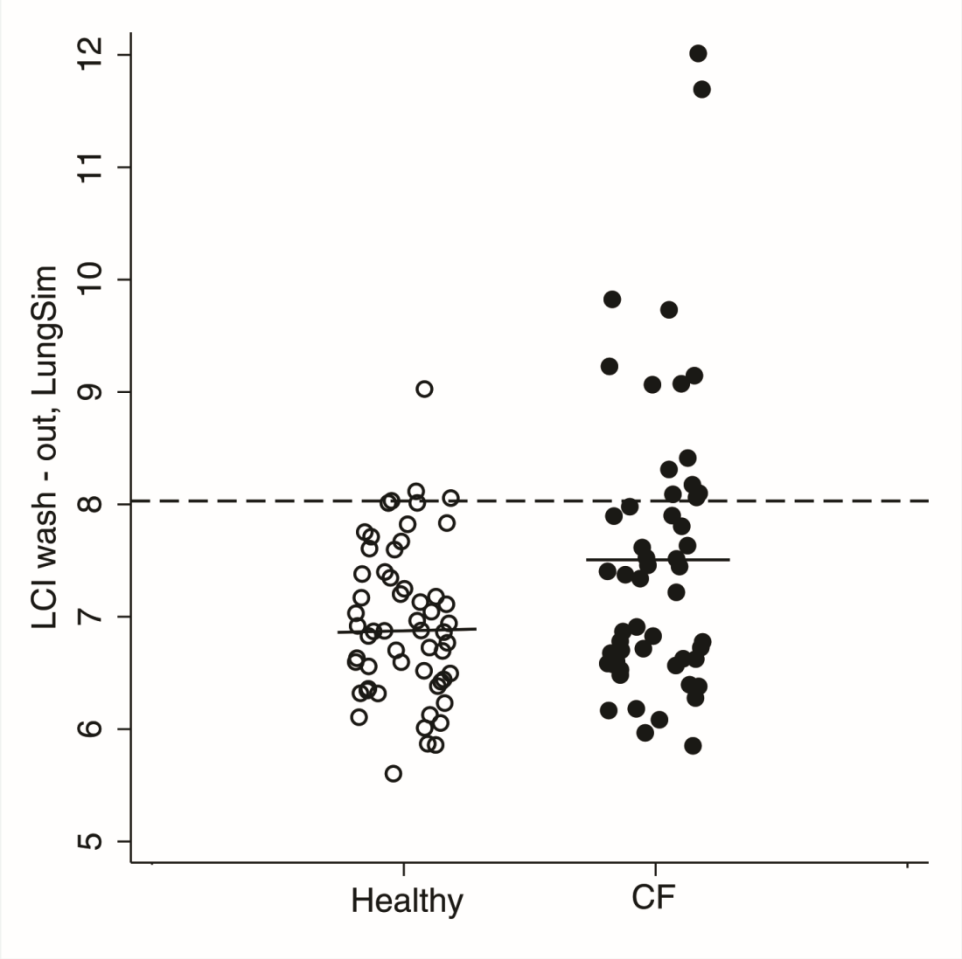

Figure S4. **Functional residual capacity of wash-out measurements analysed in the alternative software LungSim.** Functional residual capacity (FRC) and group means of sulfur hexafluoride multiple-breath wash-out in 57 healthy infants and 53 infants with cystic fibrosis (CF). Analysis were performed in the alternative software (LungSim 2.1.0, NM GmbH, Thalwil, Switzerland). The dashed line represents the upper limit of normal (mean healthy + 1.64*SD healthy).

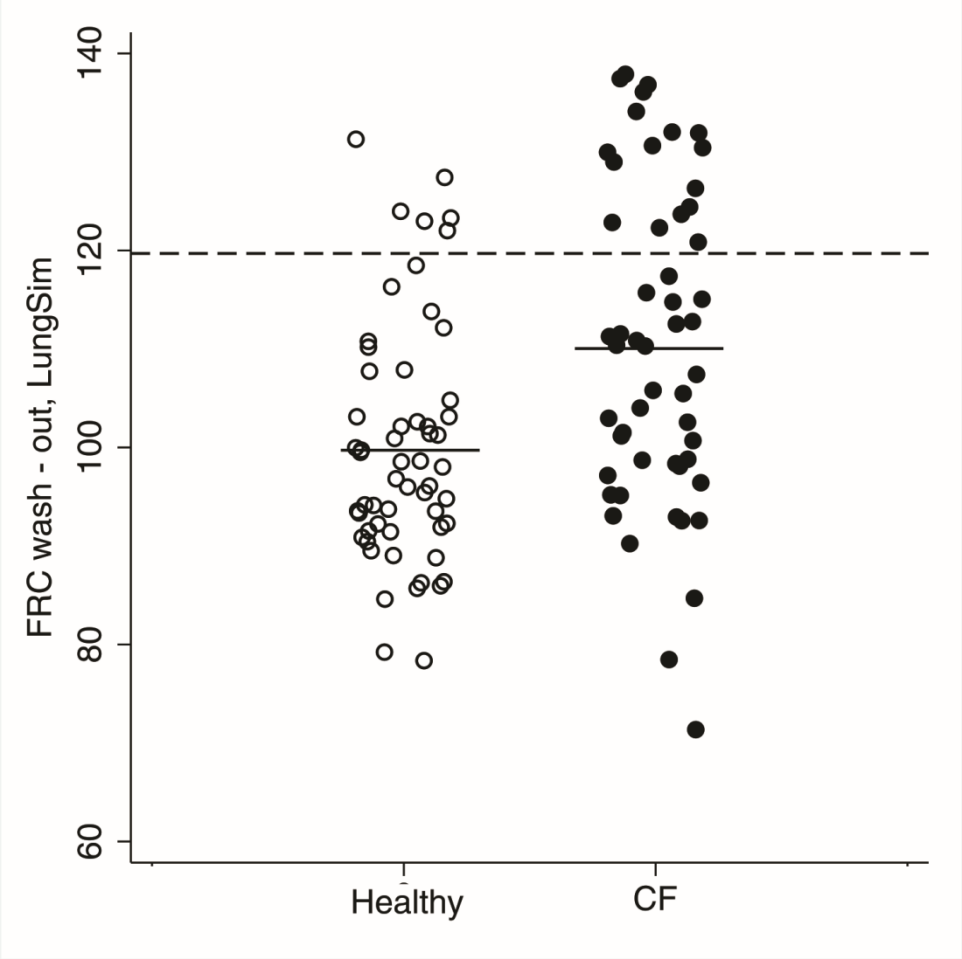

Figure S5. Standard lung clearance index in infants with cystic fibrosis and healthy controls according to the numbers of successful wash-out trials. Lung clearance index (LCI) and group means of sulfur hexafluoride multiple-breath wash-out in 57 healthy infants and 53 infants with cystic fibrosis (CF). Analysis were performed in the standard software WBreath. Open circles represent children with one valid trial (6 infants with CF, 1 healthy infant), grey circles with two valid trials (10 infants with CF, 22 healthy infants), and black circles with 3 valid trials (37 infants with CF, 34 healthy infants). The dashed line represents the upper limit of normal (7.48) calculated as mean LCI + 1.64*SD from healthy controls.

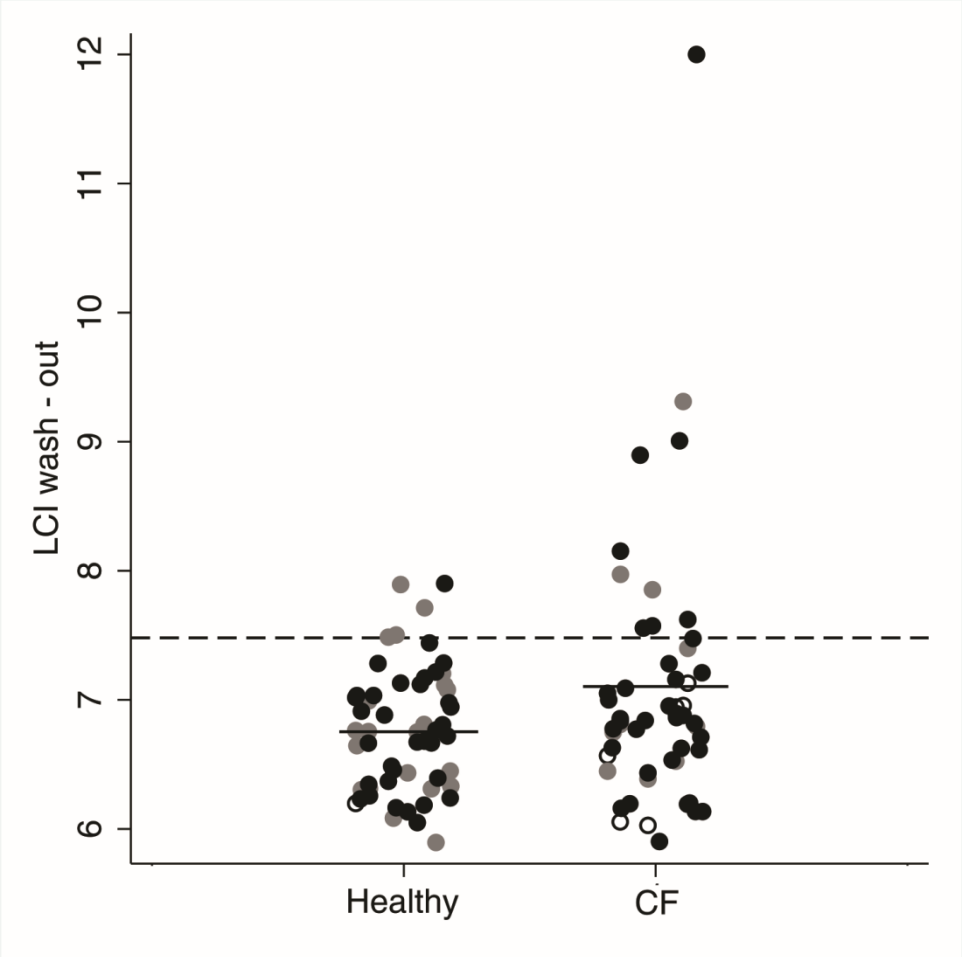

Figure S6. Standard functional residual capacity in infants with cystic fibrosis and healthy controls according to the numbers of successful wash-out trials. Functional residual capacity (FRC) and group means of sulfur hexafluoride multiple-breath wash-out in 57 healthy infants and 53 infants with cystic fibrosis (CF). Analysis were performed in the standard software WBreath. Open circles represent children with one valid trial (6 infants with CF, 1 healthy infant), grey circles with two valid trials (10 infants with CF, 22 healthy infants), and black circles with 3 valid trials (37 infants with CF, 34 healthy infants). The dashed line represents the upper limit of normal (mean healthy + 1.64*SD healthy).

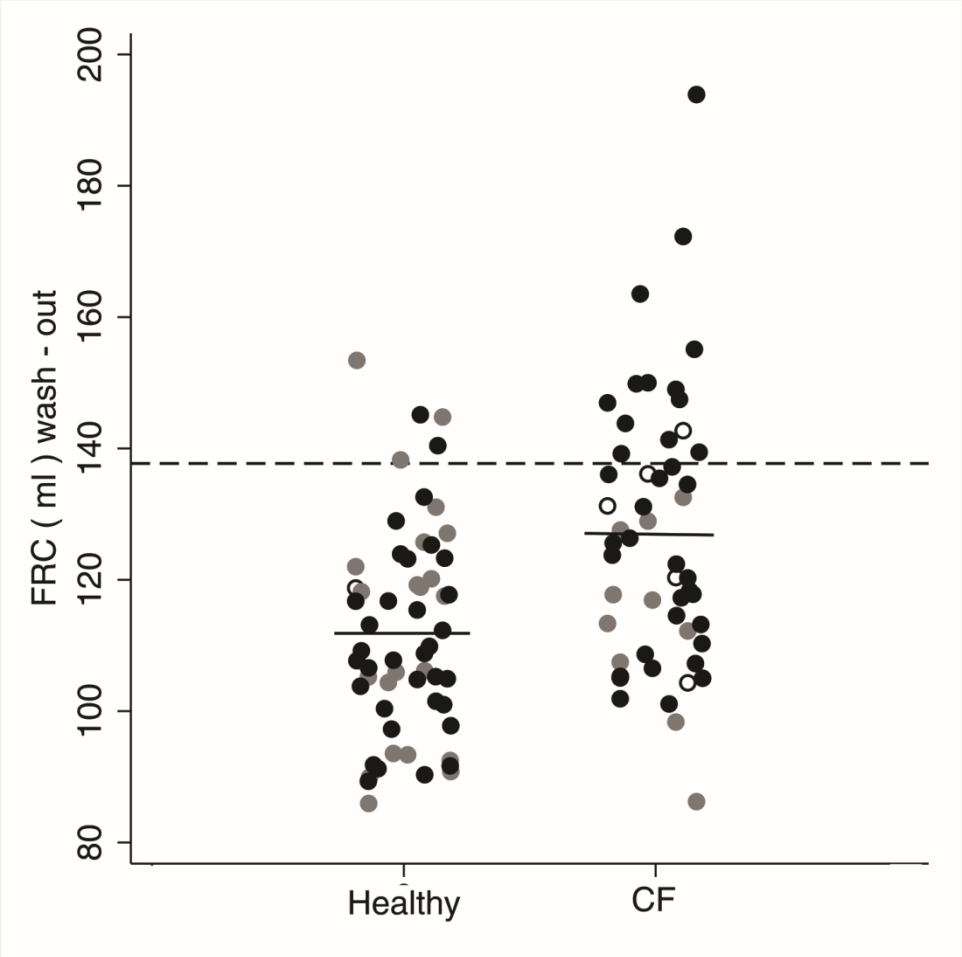

Figure S7. **Tidal volume versus weight for infants with cystic fibrosis and healthy infants.** The black line indicates the regression line with a coefficient of correlation of 0.45 for tidal volume in healthy infants and 0.69 for tidal volume in infants with CF. The respective R^2 values are given in the figure.

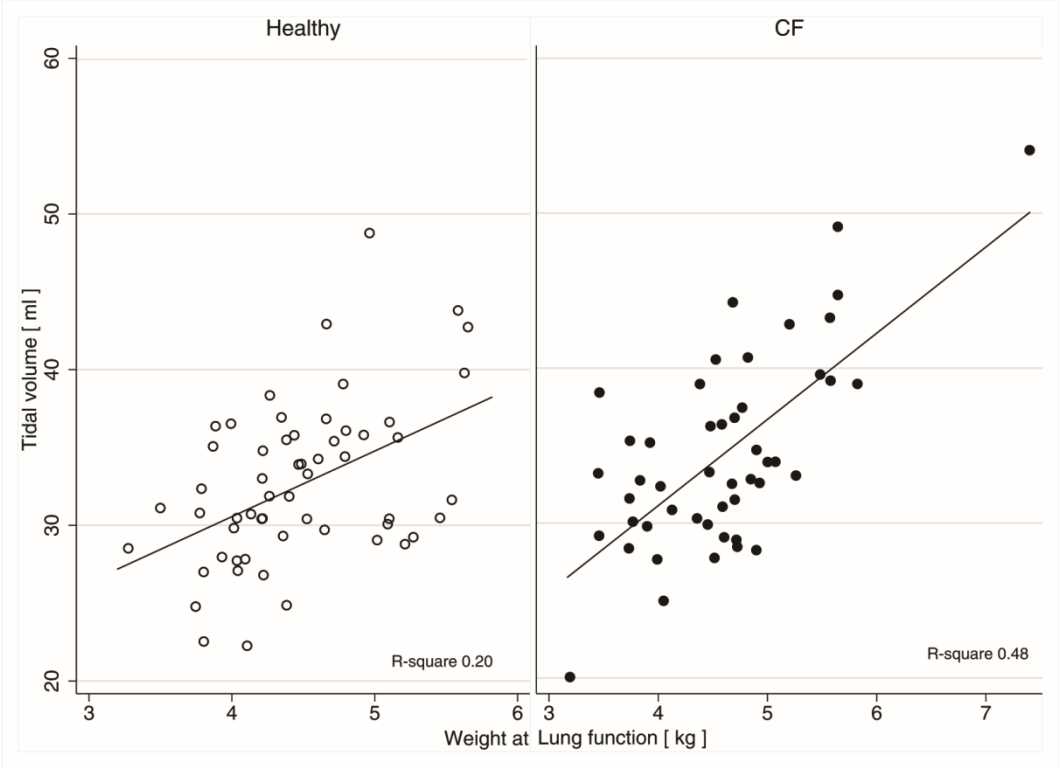

Figure S8. Comparison of lung clearance index in two subgroups of infants with cystic fibrosis. Comparison of lung clearance index (LCI) in two subgroups of infants with cystic fibrosis (CF). **(a)** The first including all infants without antibiotic treatment or respiratory symptoms (n = 42) versus all infants with antibiotic treatment and/or respiratory symptoms (n = 11) resulting in p = 0.09 (applying non-parametric Wilcoxon rank sum test). **(b)** After exclusion of one outlier with elevated LCI in the subgroup of antibiotic use and/or symptoms, comparison resulted in p = 0.20.

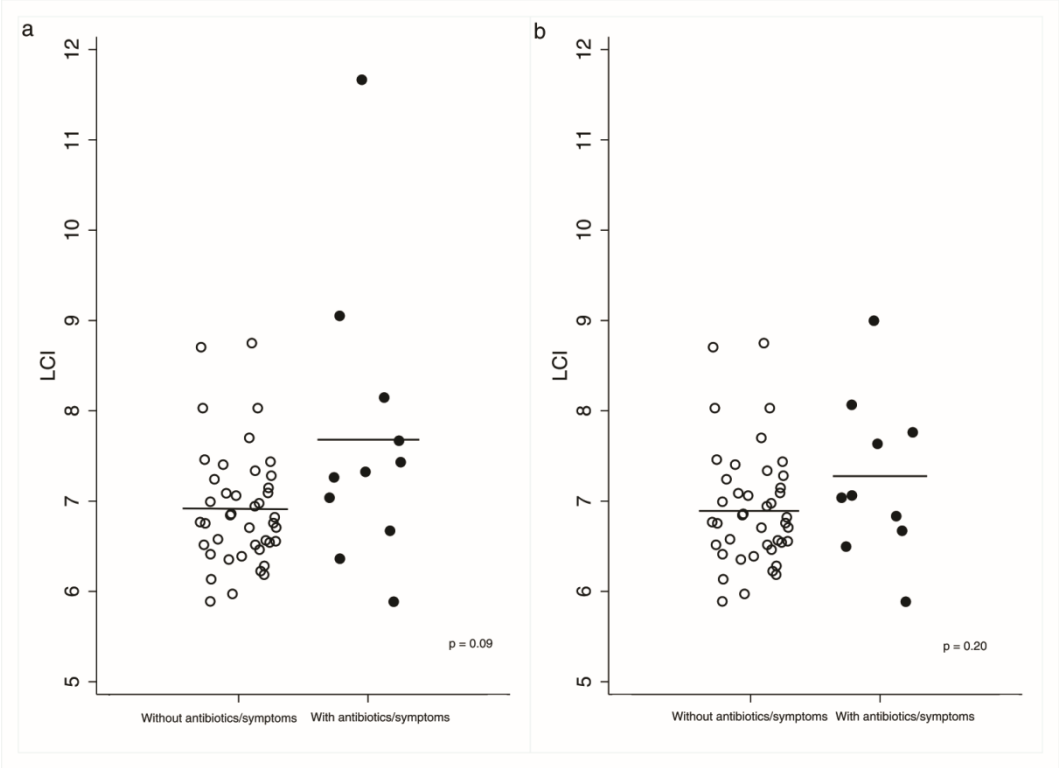

Figure S9. Association of lung clearance index and age in healthy infants. Lung clearance index (LCI) of wash-out measurements in the standard software WBreath showed no assocation with age in the 56 healthy infants.