Controlled vs free breathing for multiple breath nitrogen washout in healthy adults

Online Supplement

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1. METHODS: Participant inclusion and exclusion criteria

Inclusion:

- >18 years of age
- Free of respiratory disease
- Free of cardiovascular conditions
- No current respiratory symptoms
- No regular use of respiratory medications

Exclusion:

- Current smoking
- ≥5 pack years past smoking history
- Symptoms of respiratory tract infection in the previous 6 weeks
- Unable to provide informed written consent

2. METHODS: MBNW testing protocols

We used the Exhalyzer D with Spiroware v3.1.6 (Eco Medics AG, Duernten, Switzerland). This device measures flow via a mainstream ultrasonic flowmeter, oxygen (O_2) concentration by a side-stream laser sensor, and carbon dioxide (CO_2) by a main-stream infra-red sensor. The device measures nitrogen (N_2) concentration indirectly by subtraction of CO_2, argon and O_2 concentrations. The software accounted for the pre- and post-gas-sampling dead space (47 mL and 22 mL, respectively), and BTPS corrections.

Device calibration and quality control

Prior to each testing session, the flow sensor was calibrated using a 1 L syringe, and gas analysers were calibrated using medical air and 100 percent O2. Periodically (weekly) during the study period, gas and flow signal synchronisation was performed by a control operator breathing on the mouth piece. As a functional calibration, an “octopus” syringe lung model of known “functional residual capacity (FRC)” [1] was also tested periodically (1-4 weeks) to ensure the measured FRC was within

an acceptable range. RNSH and WIMR laboratories both conduct internal biological control programmes where 2-3 nominated healthy individuals undergo testing on a monthly basis, or as required following changes in conditions (moving of equipment, replacement of parts, software updates, etc). A standard control chart approach was used, whereby deviations from the baseline average of greater than 2xSDs were considered significant cause for investigation. There were no deviations from the expected ranges during the testing period.

Test procedure

Volunteers were asked to sit in an upright, comfortable position with bite-on rubber mouthpiece positioned securely within the mouth, whilst maintaining a tight seal with the lips and a neutral head position. The operator monitored the test using real-time flow/volume and volume/time traces. All trials started with a period of normal relaxed breathing on room air in order to establish a stable EELV as indicated on the volume-time trace. After determining that a stable breathing pattern on room air had been established, the operator switched the breathing circuit to 100% O_2. The operator visually monitored the N_2 concentration and volume/time traces to ensure there were no mouth leaks, coughs, or inspirations that exceeded the bias flow. If these were present, the operator terminated the trial and repeated it after the appropriate wash-in time (twice the length of the previous washout [2]). When the mean N_2 concentration was $1/40^{th}$ of the initial concentration, the operator asked the participant to breathe a further 5-6 breaths before terminating the trial. We considered a measurement session to be complete when there were at least 3 technically acceptable trials (i.e technically acceptable 1^{st} breath, at least 2/3 of total breaths technically acceptable, end of trial criteria met, and absence of artefacts), as per the current consensus statement [2] with FRC values within ±10% of the mean of the 3 trials.

For the free breathing protocol, the operator instructed the participant to “breathe relaxed and normally” on room air. The software’s visual incentive screen was switched off at all times during this protocol. After an initial period of breathing stabilisation (approximately 30 s), the operator commenced the washout by switching the circuit to 100% O_2 and the participant continued to breathe normally for the duration of the trial. Sometimes, at the time of acquisition, the operator observed the participant was not breathing sufficiently deeply for an adequate phase III slope to be captured. In

those cases, the operator requested that the participant breathed “a little deeper” and noted this instruction in the participant’s file.

For the controlled breathing protocol, after the initial breathing stabilisation period, the operator instructed the participant to breathe at a VT of 0.95 – 1.3 L and at RR 8-12/min with the use of the visual incentive screen as originally described by (described by Verbanck et al [3]);. Once this was achieved, and the operator was satisfied that a stable end-expiratory lung volume had been reached, they commenced the washout by switching the circuit to 100% O_2. The participant continued this same pattern of breathing until the end of the trial.

3. METHODS: MBNW analysis

We analysed MBNW data using Spiroware software (v.3.1.6).

N_2 Phase III slope (SIII) calculation

Breaths for which the operator decided there was insufficient Phase III to accurately estimate the slope were excluded. By default, the software estimates SIII as the linear regression between 50-95% of expired volume. However, the operator manually adjusted the boundaries for SIII determination where needed, e.g. to exclude Phase II (particularly when expired volume was relatively small) and when there were prominent cardiogenic oscillations.

While the test operator performed preliminary analysis on individual trials during the testing session, a single investigator re-analysed the data post hoc for all participants as a batch in order to ensure a consistent approach to analysis.

Calculation of MBNW indices

MBNW indices are calculated automatically by the Spiroware software.

- FRC is calculated as the ratio of exhaled N_2 volume to the difference in initial and final end-tidal concentrations.
- Phase III slopes, normalised for mean expired N_2 concentration within phase III for that breath (S_{nIII}), are plotted as a function of lung turnover (i.e. cumulative expired volume [CEV] divided by FRC).

- LCI is calculated by dividing the CEV measured at 1/40th of initial N_2 concentration by FRC [2].
- Scond is calculated as the slope of a linear regression of S_{nIII} between the limits of 1.5 and 6 lung turnovers.
- Sacin is calculated as S_{nIII} of the first breath minus Scond.

For the free breathing protocol, to allow comparison of Scond and Sacin between participants with different lung sizes and breathing at different VT, each SnIII is divided by FRC and then multiplied by FRC*VT of the breath, resulting in a net multiplication by VT [4]. These adjusted indices are denoted in the literature and Spiroware software as Scond*VT and Sacin*VT; however, in keeping with the nomenclature described above, we refer to them simply as $Scond_{FB}$ and $Sacin_{FB}$, respectively.

4. RESULTS: Table S1 – MBNW ventilation heterogeneity from free breathing (Z scores)

	Whole group	Repeatability subgroup
Free breathing		
Z-LCI_{FB}	1.56(1.73)	1.38(1.79)
Z-$Scond_{FB}$	-0.2(2.22)	0.12(1.76)
Z-$Sacin_{FB}$	1.15(1.88)	0.03(1.01)

Values are mean(SD). For the repeatability subgroup, results are from the first visit. Reference equations for Z-scores from the free breathing protocol were from Kjellberg 2016 [4]. These equations were derived from data collected on the same MBNW device used in the present study. LCI, lung clearance index; Scond, conductive zone ventilation heterogeneity; Sacin, acinar zone ventilation heterogeneity.

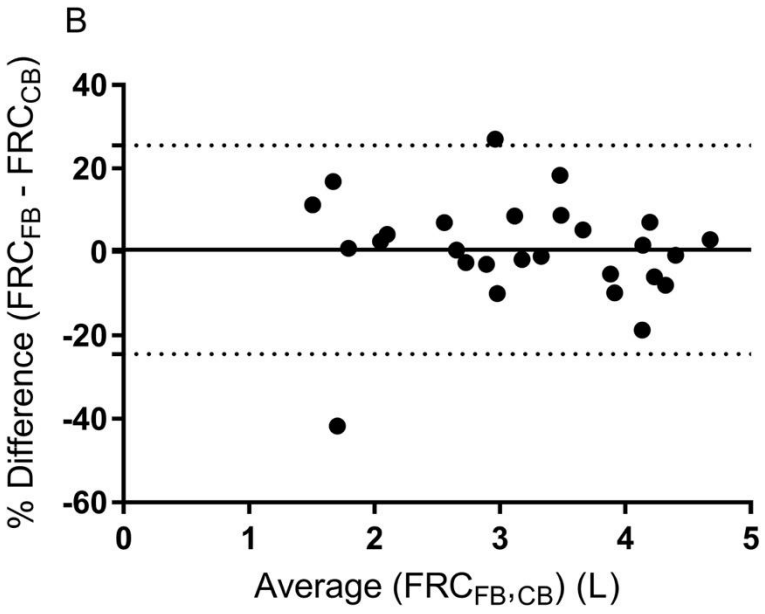
5. RESULTS: Table S2 – Scond and Sacin before and after VT correction

	Controlled Breathing	Free Breathing
Scond	0.017(0.009)	0.022(0.022)
Scond*VT	0.019(0.009)	0.018(0.01)
Sacin	0.057(0.022)	0.13(0.125)
Sacin*VT	0.064(0.023)	0.085(0.038)

Values are mean(SD). During the free breathing protocol, a correction for VT is typically made to allow comparison of Scond and Sacin between individuals with different lung sizes and breathing at different VT. These corrected indices are denoted Scond*VT and Sacin*VT. In line with the current consensus recommendations (Robinson et al 2013) [2], we are reporting both corrected and uncorrected values for both protocols. (Note: in the rest of the manuscript, Scond*VT and Scain*VT from the free breathing protocol are referred to as $Scond_{FB}$ and $Sacin_{FB}$, wheras $Scond_{CB}$ and $Sacind_{CB}$ refer to the uncorrected values from the controlled breathing protocol).

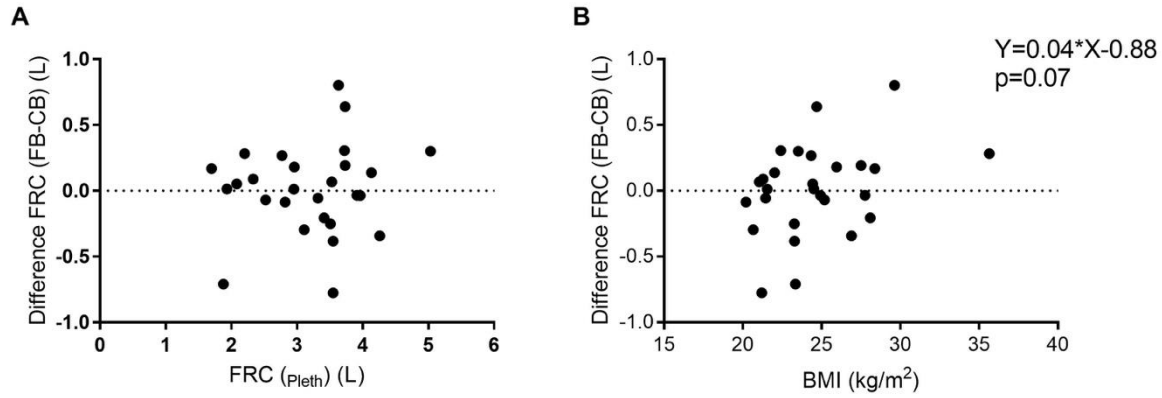
6. RESULTS: Figure S1

Figure S1. Percent difference in functional residual capacity measured by controlled breathing and free breathing protocols. Bland-Altman plot showing good agreement between the protocols (mean difference (95% limits of agreement) 0.54 (-24.43, 25.51) %, p=0.76).



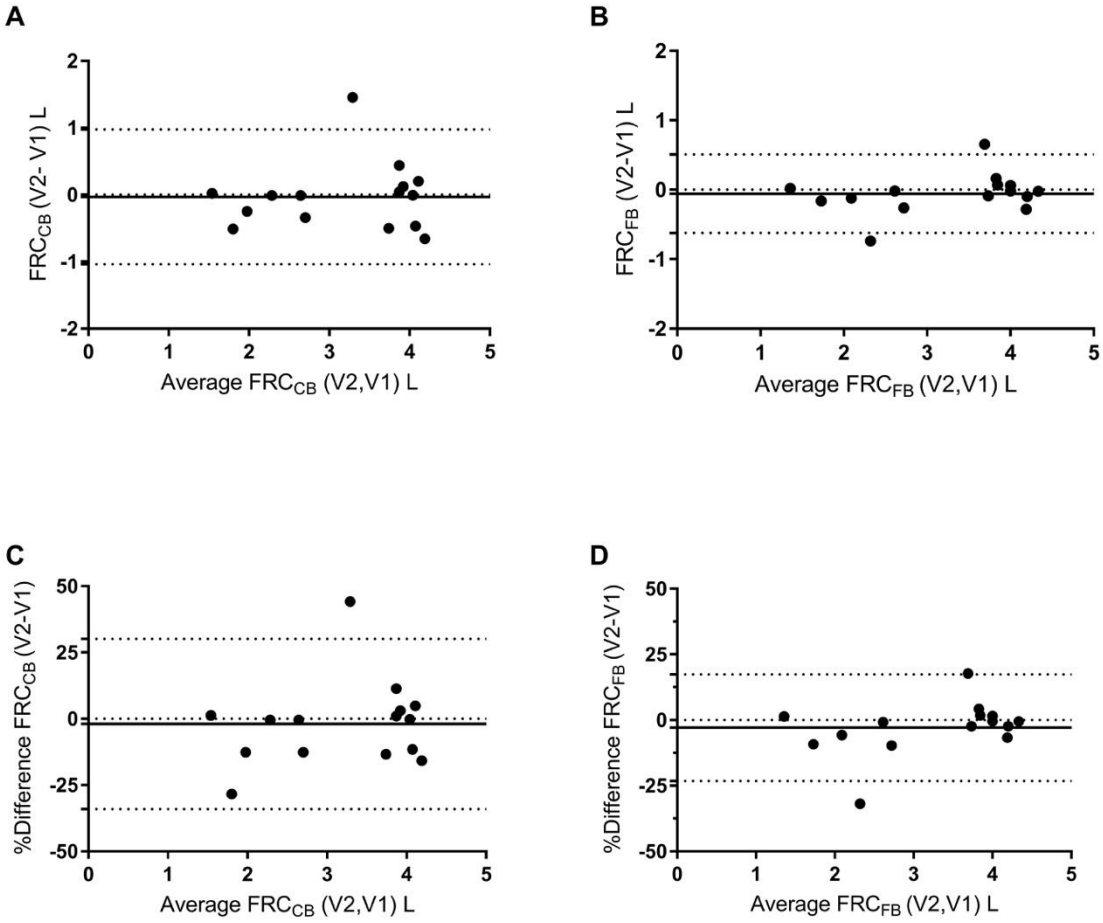
7. RESULTS: Figure S2

Figure S2. Predictors of differences in functional residual capacity measured by controlled breathing and free breathing MBNW protocols. Between-protocol differences were not predicted by FRC measured by the gold-standard body plethysmography (linear regression p=0.55) (A), but may be related to the individual's body mass index (linear regression of borderline significance, p=0.07) (B).



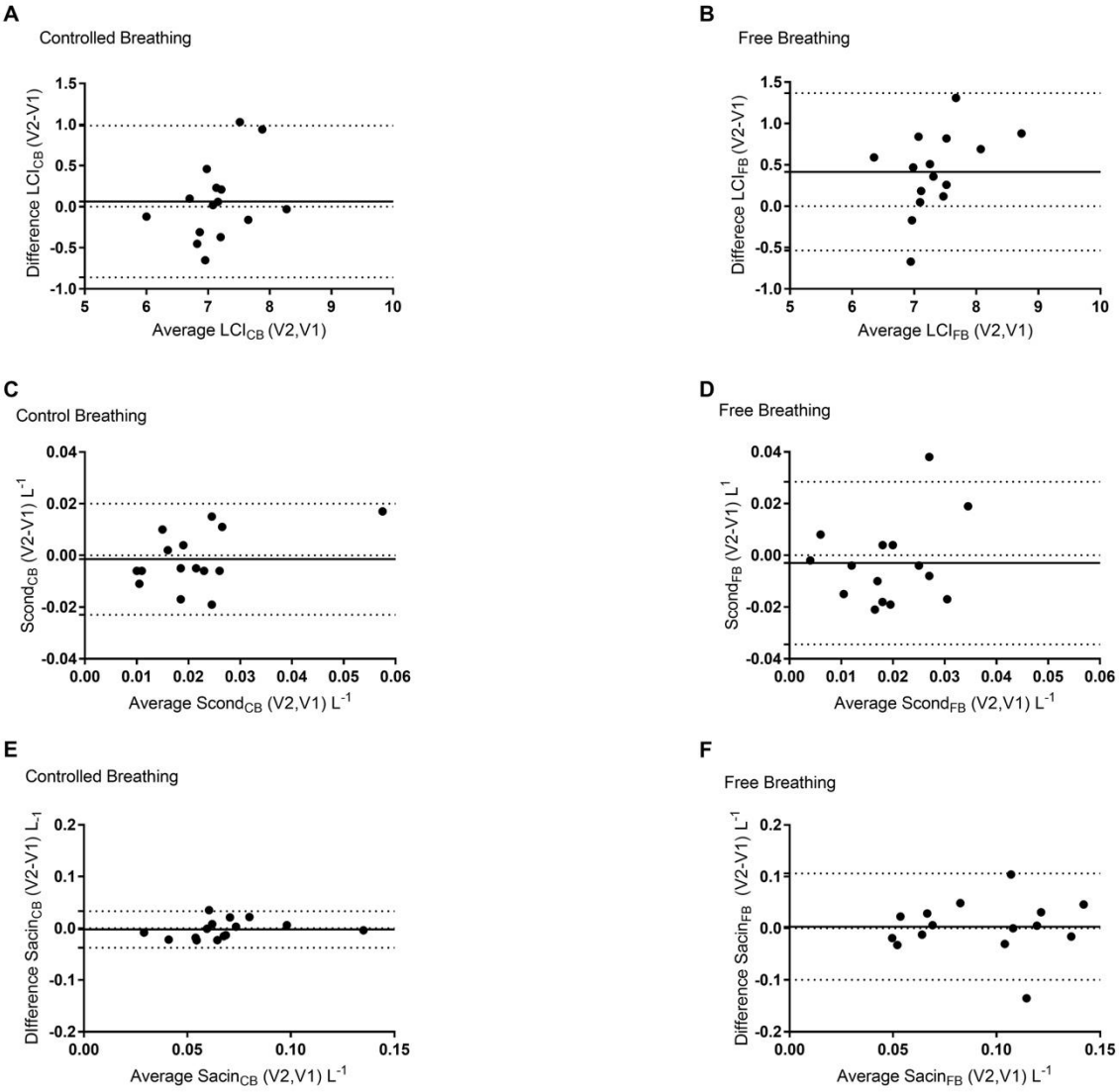
8. RESULTS: Figure S3

Figure S3. Bland-Altman plots of between-session difference (Visit 2 minus Visit 1) for functional residual capacity measured by controlled breathing (FRCCB, left panels) and free breathing (FRCFB, right panels) MBNW protocols. Mean absolute difference (95% limits of agreement [LOA]) for (A) controlled breathing -0.03 (-1.04, 0.98) L and (B) free breathing -0.05 (-0.62, 0.52). Mean percent difference (95% LOA) for (C) controlled breathing -1.98 (-34.05, 30.1) % and (D) free breathing -2.89 (-23.18, 17.41) %.



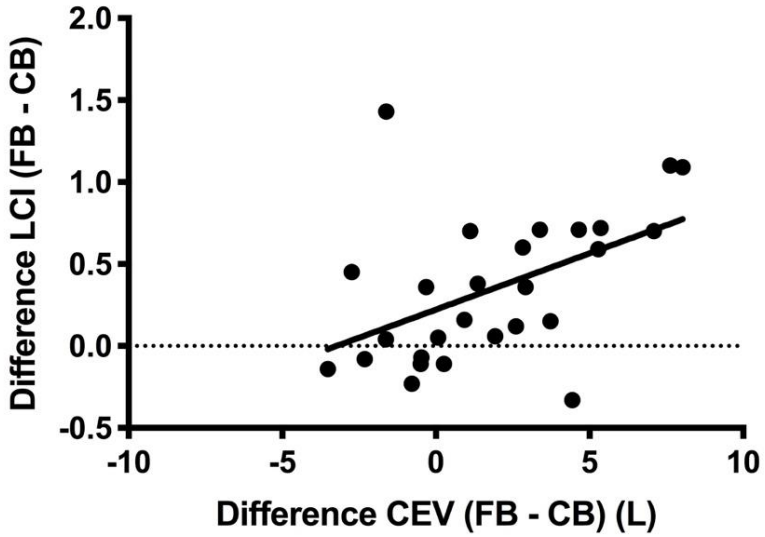
9. RESULTS: Figure S4

Figure S4: Bland-Altman plots of between-session difference (Visit 2 minus Visit 1) for ventilation heterogeneity indices measured by controlled breathing (left panels) and free breathing (right panels) MBNW protocols. Values are mean difference (95% limits of agreement). Lung clearance index (A) controlled breathing 0.06 (-0.86, 0.98) and (B) free breathing 0.42 (-0.54, 1.37). Scond (C) controlled breathing -0.001 (-0.017, 0.015) and (D) free breathing 0.003 (-0.027, 0.033). Sacin (E) controlled breathing -0.003 (-0.037, 0.031) and (F) free breathing 0.004 (-0.098, 0.106).



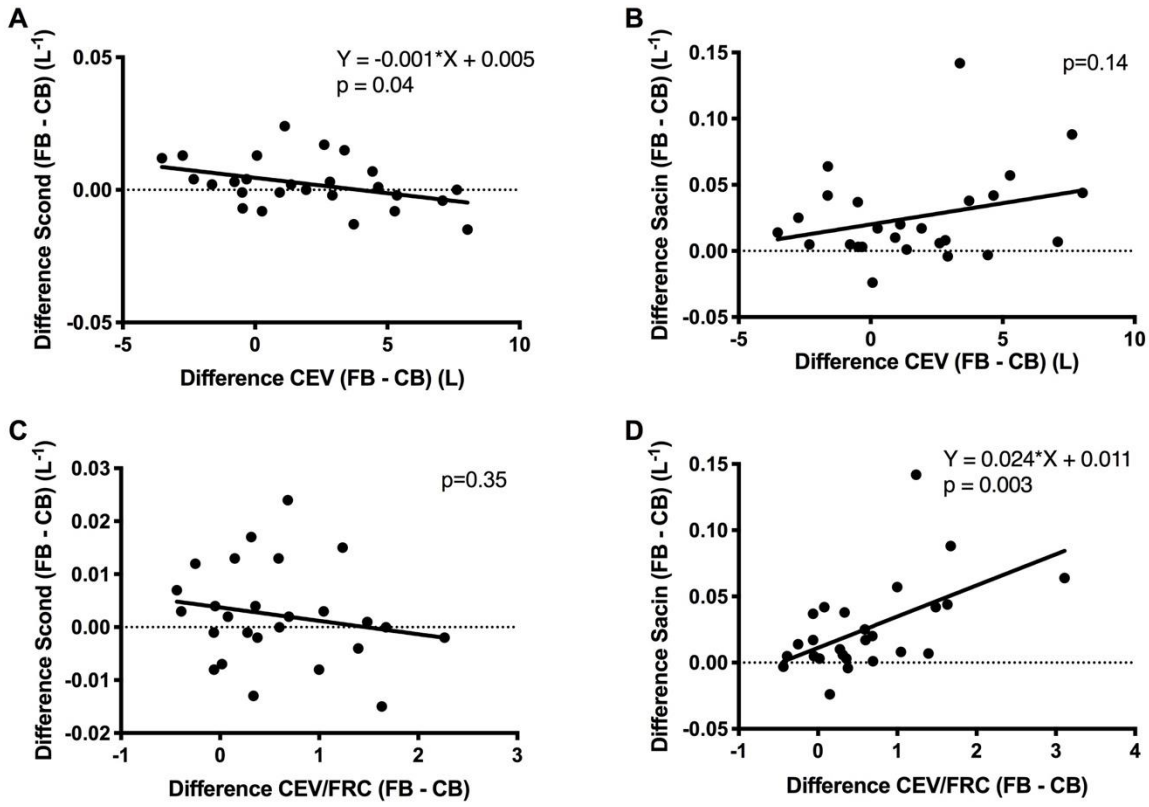
10. RESULTS: Figure S5

Figure S5: Relationship between differences in functional residual capacity (FRC) and change in cumulative expired volume (CEV) between controlled breathing and free breathing protocols.



11. RESULTS: Figure S6

Figure S5: Between-protocol differences in Scond and Sacin plotted against differences in cumulative expired volume (CEV, top panels) and CEV corrected for functional residual capacity (FRC, bottom panels). Between-protocol difference in Scond was related to between-protocol difference in CEV (linear regression p=0.04) (A) but not CEV/FRC (p=0.35) (C). Between-protocol difference in Sacin was not related to difference in CEV (p=0.14) (B) but was significantly related to CEV/FRC (p=0.003) (D). n=1 outlier excluded from Scond analyses. n=1 participant excluded from Sacin analyses due to negative value in one trial.



12. REFERENCES

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