

Factors influencing the assessment of lung function in mice with influenza-induced lung disease

Alexander N. Larcombe,^a Graeme R. Zosky,^a Cindy Thamrin,^b Elizabeth M. Bozanich,^a Zoltán Hantos,^{a,c} Peter D. Sly^d

^aDivision of Clinical Sciences, Telethon Institute for Child Health Research & Centre for Child Health Research, University of Western Australia, Perth, WA, Australia. ^bAirway Physiology/Imaging Research Group, Woolcock Institute of Medical Research, Sydney, NSW, Australia. ^cDepartment of Medical Physics and Informatics, University of Szeged, Szeged, Hungary. ^dQueensland Children's Medical Research Institute, University of Queensland, Brisbane, Qld, Australia.

Correspondence: Alexander Larcombe, Division of Clinical Sciences, Telethon Institute for Child Health Research, PO Box 855, West Perth, WA 6872, Australia.

E-mail: alexanderl@ichr.uwa.edu.au

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Background The constant-phase model (CPM) is commonly fit to respiratory system input impedance (Z_{rs}) to estimate lung mechanics. Driving signal frequencies and the method of model fitting may influence the results, especially in cases of severe lung disease or under severe bronchoconstriction.

Objective To illustrate the effects of different CPM fits to Z_{rs} data using a mouse model of influenza-induced lung disease.

Methods BALB/c mice infected with influenza (or control) were challenged with methacholine. The CPM was fitted to Z_{rs} measured between 0.25 and 19.625 Hz, using both unweighted

and weighted fits. The effect of different lowest frequencies was assessed.

Results and Conclusions For influenza-infected mice, the unweighted fit was poor, and airway resistance (R_{aw}) was often biologically impossible. The weighted fit provided more realistic estimates of R_{aw} . Different model fits and minimal frequencies had little effect on tissue mechanics.

Keywords Constant-phase model, forced oscillation technique, influenza, mouse, severe lung disease.

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Introduction

Measurement of lung function forms an important part of the use of animals to model human lung diseases. The effects of respiratory system insults are often obtained through fitting mathematical models, such as the constant-phase model (CPM), to respiratory system impedance (Z_{rs}). The CPM partitions low-frequency input Z_{rs} into components representing airway mechanics, that is, airway resistance (R_{aw}) and inertance (I_{aw}), and a strictly constant-phase tissue compartment characterized by the coefficients of tissue damping (G) and tissue elastance (H).¹ The CPM has been validated in a variety of species,^{1,2} and its sensitivity has been assessed in mice.³ The relative contributions of the airway and tissue compartments to Z_{rs} within a given frequency range may change radically if any alterations occur predominantly in either compartment, such that the other compartment will be subjected to estimation bias. This may occur during acute bronchoconstriction where

the contributions from the airway may increase markedly or in severe lung disease where the tissue component may dominate. It may be possible to alleviate the estimation bias by studying a more appropriate frequency range or by using different methods for fitting the CPM to Z_{rs} .³

We investigated the influence of the frequency content of the driving signal and the method used to fit the CPM to Z_{rs} on estimates of R_{aw} , G , and H in mice with influenza-induced lung disease.

Methods

Eight-week-old female BALB/c mice, maintained under specific pathogen-free conditions and with food and water provided *ad libitum*, were intranasally inoculated with $10^{4.5}$ plaque-forming units of influenza A/Mem/1/71 diluted in 50 μ l of virus production serum-free medium (VP-SFM; Gibco, Mulgrave, Victoria, Australia). Seven mice were infected (mass 17.6 ± 1.1 g), and seven control mice (mass

18.7 ± 0.5 g) received the same volume of the supernatant of uninfected Madin–Darby canine kidney cells diluted in VP-SFM. Influenza was grown and titrated as previously described.⁴ This strain replicates to high titres⁴ and causes significant disease in mice.⁵

At the peak of infection,⁶ mice were surgically prepared as described previously.⁵ Anaesthetized, tracheostomized mice were ventilated using a flexiVent small animal ventilator (SCIREQ, Montreal, Canada) at 450 breaths per min with a tidal volume of 8 ml/kg and a positive end-expiratory pressure of 2 cm H₂O. Lung volume history was standardized via three slow (16-s) deep inflations, spaced 1 min apart, to a pressure of 20 cm H₂O and Z_{rs} measured 1 min after increasing doses (0.1, 0.3, 1, 3, 10, and 30 mg/ml) of aerosol-

ized methacholine (MCh; Sigma-Aldrich, St. Louis, MO, USA) generated by an ultrasonic nebulizer (DeVilbiss Ultra-Neb, Somerset, PA, USA).^{5,7} We report measurements at baseline (i.e., before any MCh) and after 30 mg/ml MCh.

Z_{rs} was measured using the “Primewave” signal of the flexiVent small animal ventilator, which is comprised of 19 mutually prime sinusoidal frequencies (0.25, 0.625, 1.375, 2.125, 2.875, 3.875, 5.125, 5.875, 7.375, 8.375, 9.125, 10.375, 12.125, 12.875, 13.625, 15.875, 17.125, 18.625, and 19.625 Hz). The CPM¹ was used to calculate Newtonian resistance (R_n), I_{aw} , G , and H . Hysteresivity (η) was calculated as G/H . We assumed that the contribution of the lung parenchyma and the chest wall to R_n was insignificantly small⁸ and hence $R_n = R_{aw}$. I_{aw} values were

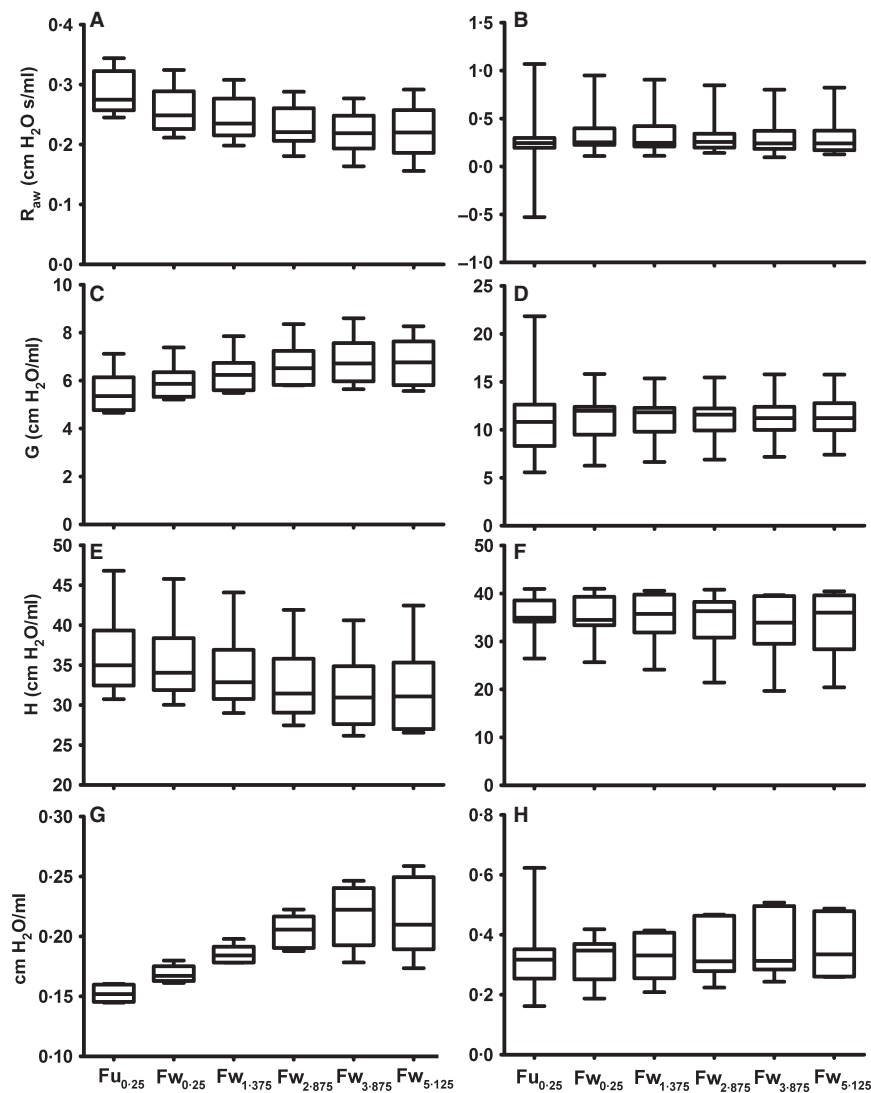


Figure 1. Box and whisker plots of baseline airway resistance (R_{aw}), tissue damping (G), tissue elastance (H), and hysteresivity (η) of BALB/c mice, 4 days post-inoculation with influenza A (B, D, F and H) or control solution (A, C, E and G) using different methods of fitting the constant-phase model to Z_{rs} spectra. Note different scales.

negligible and are not reported. Z_{rs} data were imported into custom-made analysis software (University of Szeged, Hungary) for analysis, which uses a global optimization algorithm⁹ to fit the CPM to Z_{rs} . Data were initially analyzed using the complete set of 19 frequencies using an unweighted fitting criterion:

$$\Phi_{\text{abs}} = \left[\frac{1}{n} \sum_{i=1}^n |Z_d(f_i) - Z_m(f_i)|^2 \right]^{\frac{1}{2}}$$

where Φ is the fitting error, and $Z_d(f_i)$ and $Z_m(f_i)$ correspond to the measured and model-predicted impedance value at the i th frequency, respectively.³ The CPM was then fitted to the measured data using a weighted fitting

criterion, where non-negative weights were incorporated into the fitting criterion:

$$\Phi_{\text{rel}} = \left[\frac{1}{n} \sum_{i=1}^n \frac{|Z_d(f_i) - Z_m(f_i)|^2}{|Z_d(f_i)|^2} \right]^{\frac{1}{2}}$$

The weighted fitting criterion scales the contribution of each frequency by the magnitude of the impedance at that frequency, such that model fit is not dominated by frequency points where Z_{rs} is larger compared to other frequencies. Z_{rs} data were then adjusted to alter the lowest frequency, and the model was refit using the weighted fitting criterion, such that we had one set of data which was analyzed in six ways;

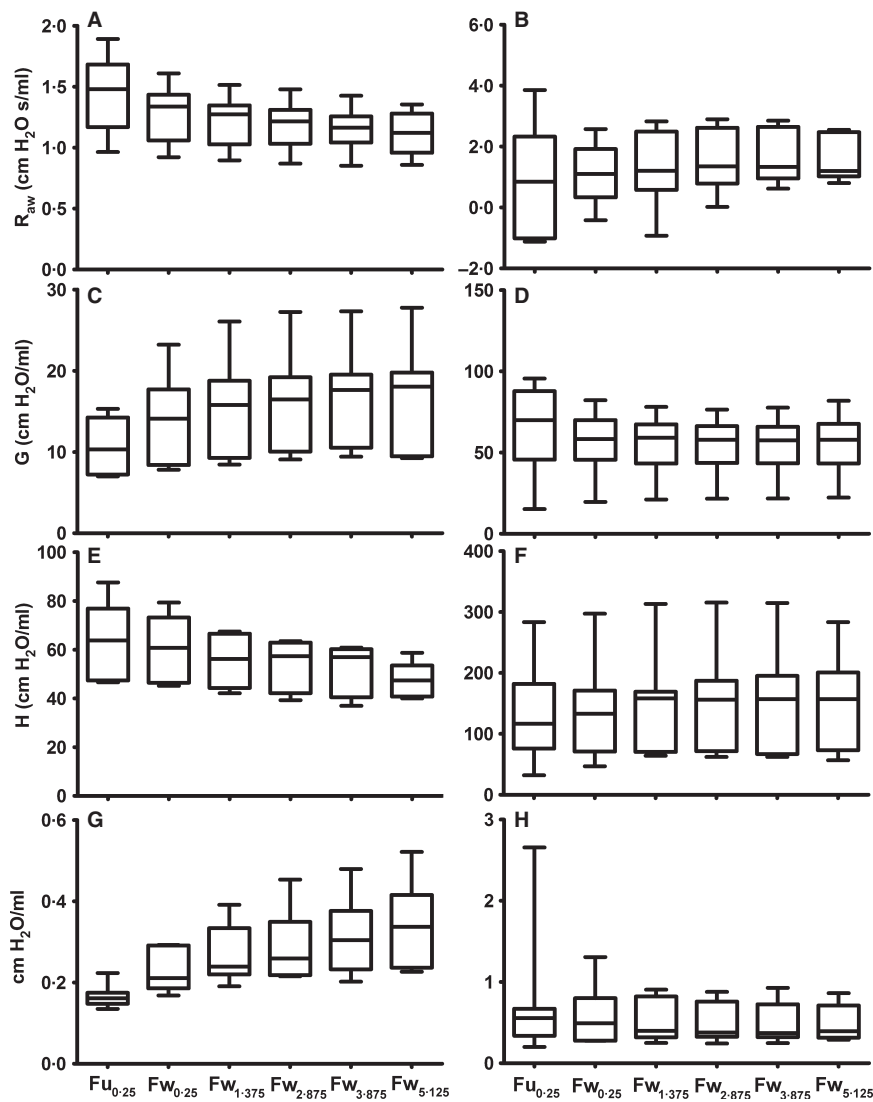


Figure 2. Box and whisker plots of maximum (after exposure to 30 mg/ml MCh) airway resistance (R_{aw}), tissue damping (G), tissue elastance (H), and hysteresivity (η) of BALB/c mice, 4 days post-inoculation with influenza A (B, D, F and H) or control solution (A, C, E and G) using different methods of fitting the constant-phase model to Z_{rs} spectra. Note different scales.

1. $Fu_{0.25}$: unweighted fit/0.25–19.625 Hz,
2. $Fw_{0.25}$: weighted fit/0.25–19.625 Hz,
3. $Fw_{1.375}$: weighted fit/1.375–19.625 Hz,
4. $Fw_{2.875}$: weighted fit/2.875–19.625 Hz,
5. $Fw_{3.875}$: weighted fit/3.875–19.625 Hz, and
6. $Fw_{5.125}$: weighted fit/5.125–19.625 Hz.

Results

Baseline Lung Function

Applying a weighted CPM fit to Z_{rs} spectra (compared to an unweighted fit) had the greatest influence on estimates of R_{aw} at baseline, and this effect was more pronounced for influenza-infected mice compared to controls (Figure 1). For infected mice, the unweighted fit resulted in the lowest and most variable estimation of R_{aw} (0.25 ± 0.46 cm H₂O s/ml) for any fitting criterion. This was a reflection of the CPM returning a negative (and biologically impossible) R_{aw} (-0.53 cm H₂O s/ml) for one of the seven mice in this group when the $Fu_{0.25}$ was used. Applying the $Fw_{0.25}$ fitting scheme to this measurement resulted in a more biologically realistic R_{aw} (0.39 cm H₂O s/ml). This skewing of R_{aw} using the $Fu_{0.25}$ criterion had the flow on effect of increasing G for infected mice. Employing a weighted fitting of the CPM for influenza-infected mice resulted in a reduction in the SD of baseline R_{aw} and G , regardless of the frequency range used. At baseline, increasing the lowest

frequency of the driving signal had little effect on estimates of, or variability in, measurements of R_{aw} , G , or H beyond the difference made by applying the $Fw_{0.25}$ (Figure 1).

Maximum response to MCh (30 mg/ml)

A similar pattern was noted for R_{aw} after exposure to 30 mg/ml MCh (Figure 2). Again, when using the $Fu_{0.25}$ fitting criterion, R_{aw} for infected mice was underestimated due to three of the seven mice in this group returning a negative estimation of R_{aw} . Applying the $Fw_{0.25}$ fitting scheme resulted in non-negative estimates of R_{aw} for two of these mice; however, it was not until the minimum frequency was increased to 1.375 Hz that six of the seven infected mice returned biologically realistic estimates of R_{aw} (Figure 2). The seventh mouse required a minimum driving signal frequency of 5.125 Hz before a realistic estimate of R_{aw} was achieved. Considerable alterations in estimates of G and H were also observed when using the $Fw_{0.25}$ compared to the $Fu_{0.25}$; however, increasing the minimum driving signal frequency beyond 1.375 Hz resulted in little change in these measurements or their variability (Figure 2). Representative impedance spectra and CPM fit for an influenza-infected mouse after exposure to 30 mg/ml MCh are shown in Figures 3 ($Fu_{0.25}$) and 4 ($Fw_{1.375}$). These figures illustrate the improved fit of the CPM to Z_{rs} , particularly in panels C.

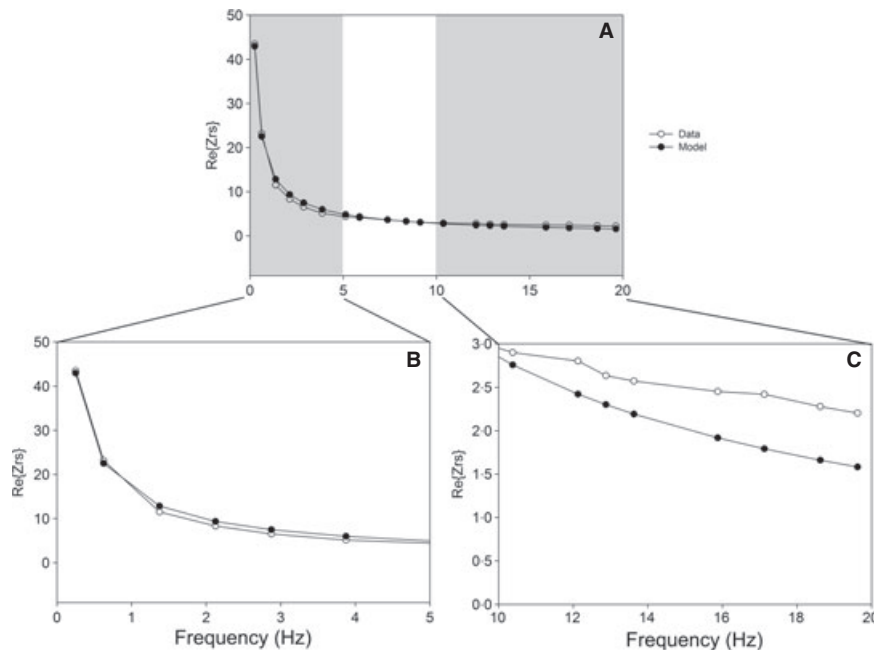


Figure 3. Representative “real part” of the impedance spectrum and constant-phase model (CPM) fit for an influenza-infected mouse after exposure to 30 mg/ml MCh, using the $Fu_{0.25}$: unweighted fit/0.25–19.625 Hz criterion. Panel A shows the entire Z_{rs} , while panels B and C zoom in on either end of the Z_{rs} . For this individual, $R_{aw} = -0.73$ cm H₂O s/ml, $G = 47.25$ cm H₂O/ml, and $H = 90.61$ cm H₂O/ml. Open circles represent Z_{rs} measured at each frequency, and closed circles represent CPM fit. The data shown in Figure 4 are for the same measurement from the same mouse, using an alternative fit criterion.

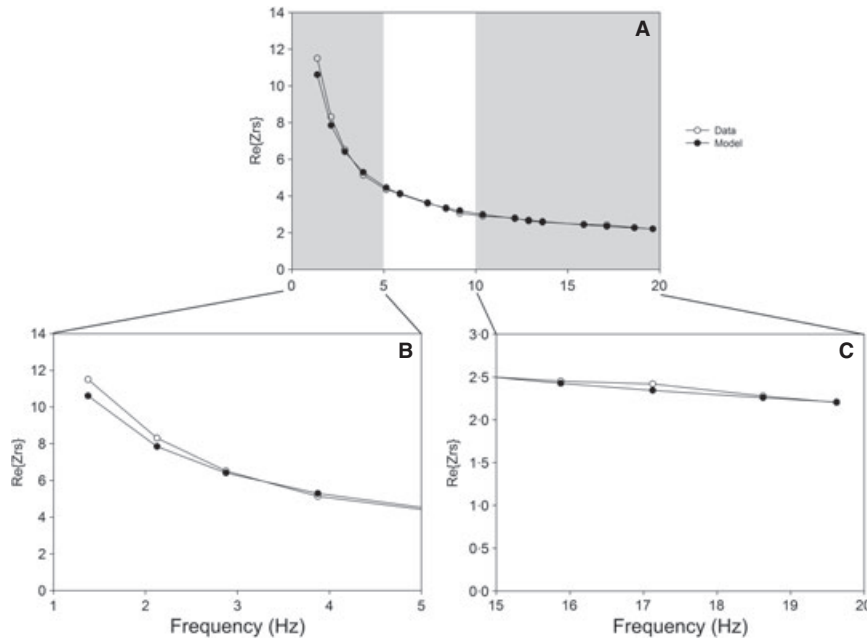


Figure 4. Representative “real part” of the impedance spectrum and constant-phase model (CPM) fit for an influenza-infected mouse after exposure to 30 mg/ml MCh, using the $Fw_{1.375}$: weighted fit/1.375–19.625 Hz criterion. Panel A shows the entire Z_{rs} , while panels B and C zoom in on either end of the Z_{rs} . For this individual, $R_{aw} = 0.85$ cm H₂O s/ml, $G = 37.31$ cm H₂O/ml, and $H = 121.57$ cm H₂O/ml. Open circles represent Z_{rs} measured at each frequency, and closed circles represent CPM fit. The data shown in Figure 3 are for the same measurement from the same mouse, using an alternative fit criterion.

Discussion

This study illustrates the importance of selecting appropriate settings for the measurement of lung mechanics when applying the CPM to Z_{rs} spectra. The greatest differences were found when comparing an unweighted ($Fu_{0.25}$) fit of the CPM to Z_{rs} spectra with any of the weighted fitting criteria. Applying a weighted fit of the CPM to Z_{rs} spectra can improve measures of lung mechanics under acutely bronchoconstricted conditions by preventing undue contributions from the lower frequencies, which have intrinsically larger magnitudes. In severe lung disease, such as that caused by influenza infection, Z_{rs} may increase even further at low frequencies, and the negative frequency dependence of the real part of Z_{rs} may strengthen^{10–12} leading to misestimation of R_{aw} . We have also shown that selecting different lowest frequencies of the forced oscillation signal can have considerable impacts on the estimation of CPM parameters. Generally, we identified slight differences in the model fit with increasing lowest frequency, up to a point where the potential benefits of the removal of additional low frequencies were outweighed by the loss of degrees of freedom of the model.

Our data show that the frequencies of a forced oscillation technique signal should be carefully selected so that the measured Z_{rs} as a result of the tissues and airways has an equal “opportunity” to contribute to parameters speci-

fied in the model. Selection of an appropriate frequency range is dependent on multiple factors including species and disease state. Under the particular set of circumstances employed in the current study (i.e., mice with influenza), an unweighted fit and frequency range of 1.375–19.625 Hz provided the most realistic measurements. When undertaking studies that apply the CPM to Z_{rs} spectra, researchers need to be aware that fitting criteria and frequency selection are important. Without previous knowledge of what frequencies are appropriate, a useful starting point may be the selection of the lowest frequency based on the resting respiratory rate of the study species. For example, the laboratory mouse has a resting breathing rate (f_R) \sim 80 to 200 breaths per minute.¹³ Thus, the lower end of the forced oscillatory signal, for an animal this size, should be \sim 1.3 to 3.3 Hz. This relationship holds for larger species, such as humans with a resting f_R of 15 breaths per min (0.25 Hz); indeed, many studies on low-frequency respiratory mechanics in humans select similar frequencies.^{14,15} In saying this, some researchers employ much lower minimum frequencies (e.g., 0.078 Hz) in studies of broncho-constriction and acute lung injury.^{16,17} The selection of the highest frequency is perhaps not as vital, although they are usually within the medium frequency range (4–40 Hz)^{18,19}, which means that resonance and anti-resonance phenomena^{19,20} are not examined in low-frequency studies.

Acknowledgements

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